Electronic Supplementary Information (ESI)

Non-ionic self-assembling amphiphilic polyester dendrimers as new drug delivery excipients

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1. Experimental section- Supporting information

1.1 In vitro cytotoxicity

The GMS and GMOA ADs were evaluated for their biocompatibility by in vitro cytotoxicity through a MTT assay.[1] Breast adenocarcinoma (MCF 7), human liver hepatocellular carcinoma (Hep G2) and human lung carcinoma (A549) cell lines were cultured in complete medium at 37 ^{o}C in humidified atmosphere of 5% in air. All cell lines harvested in the exponential phase were seeded equivalently (2.2 X 10^3) in a 96 well plate and allowed to adhere by incubating for 24 h. Thereafter, the culture media was replaced with 100 µl fresh medium and sample stock solution in distilled water was added to the medium to achieve concentrations of 20, 40, 60, 80 and 100 µg/ml. The wells containing only medium were considered as controls and wells with only media without cells were considered as blank. All experiments were performed in four replicates. After incubation for 48 h, the culture media with compounds were removed and replaced with 100 µl fresh media and 100 µl MTT solution (5 mg/ml in PBS) for each well and again incubated for 4 h. After 4 h of incubation,

the media and MTT solution were removed and replaced with 100 µl of DMSO to solubilize MTT formazan. Optical density (OD) of each well was determined at wavelength of 540 nm using a microplate spectrophotometer (Spectrostar nano, Germany). Percentage cell viability was calculated using the following equation.

% cell survival = (A540 nm treated cell / A540 nm untreated cells) X 100

1.2 HPLC method for determination of FSD[2]

Reverse phase C18 column (Nucleosil 120-5 C18; 4 x 150 mm, 5 µm) was used and the mobile phase was consists of a 3:1 mixture of methanol and 0.01 M Potassium hydrogen phosphate. The column temperature, flow rate, injection volume and detection wavelength were 25 ^{o}C, 2 ml/minute, 20 µL and 254 nm respectively. The retention time (RT) for FSD was 7.1 ± 0.19. The standard calibration curve was determined within concentration rage of 5 to 30 µg/ml.

1.3 Determination of %EE and percentage drug loading (%DL)

The %EE of FSD loaded ADs micelles was determined as per reported cetrifugal-ultrafiltration mehtod.[3] Brifly 1 ml of FSD loaded ADs micelles were added into Amicon® Ultra-4, centrifugal filter tubes (MWCO10 kDa, Millipore Corp., USA) and centrifuged for 30 min at 5000 rpm. The ammount of FSD in ultrafiltrate was determined by HPLC method as per previous section. The total ammount of FSD was determined by dissolving FSD loaded ADs micelles in to methonol and HPLC analysis of it. %EE and %DL of FSD loaded micelles was determined by following equation.

$\%EE = C_0 - C / C_0 \times 100$

$\%DL = C_0 - C / C_o - C + C_m \times 100$

Where C is the FSD concentration in ultrafiltrate, C_0 is the concetration of FSD in drug loaded micelles and C_m is the concetration of ADs in the micelles.

2. Results section – Supporting information

Table S1. Elemental analysis of ADs.

AD	Observed values		Theoretical values	
	C [%]	H [%]	C [%]	H [%]
GMS-G2-OH	57.31	8.62	58.05	8.60
GMS-G3-OH	55.08	8.06	55.09	7.82
GMOA-G2-OH	55.88	8.64	58.16	8.42
GMOA-G3-OH	54.62	7.87	55.14	7.73

Table S2. Effect of FSD solubilization on size, PDI and ZP of agregates of amphiphiles.

Formulations	**Size (nm)**	**PDI**	**ZP (mV)**
GMS-G2-OH	7.6 ± 0.13	0.26 ± 0.05	-2.61 ± 0.12
FSD-GMS-G2-OH	22.15 ± 3.5	0.75 ± 0.03	-16 ± 0.52
GMS-G3-OH	6.48 ± 0.04	0.62 ± 0.06	-0.01 ± 0.05
FSD-GMS-G3-OH	6.48 ± 0.71	0.20 ± 0.04	-12.6 ± 1.5
GMOA-G2-OH	8.62 ± 0.48	0.27 ± 0.03	-5.5 ± 0.76
FSD-GMOA-G2-OH	7.2 ± 0.09	0.34 ± 0.02	-15.5 ± 0.4
GMOA-G3-OH	12.38 ± 0.36	0.34 ± 0.02	-7.9 ± 0.763
FSD-GMOA-G3-OH	12.55 ± 1.18	0.35 ± 0.07	-7.9 ± 1.02
PF-68	28.67 ± 4.87	0.716 ± 0.02	-2.39 ± 0.40
FSD-PF-68	142.6 ± 9.86	0.625 ± 0.06	-2.91 ± 0.57

NA = Not applicable

Table S3. Amount of unentrapped, entrapped FSD and %EE of micelles from ADs

ADs	Total FSD content (µg/ml)	Unentrapped FSD (µg/ml)	Entrapped FSD (µg/ml)	%EE	%DL
GMS-G2-OH	29.0 ± 3.4	26.7 ± 0.5	2.3 ± 0.5	8.0 ± 1.9	0.023 ± 0.005
GMS-G3-OH	21.9 ± 1.7	20.9 ± 0.2	1.0 ± 0.2	4.5 ± 0.9	0.01 ± 0.002
GMOA-G2-OH	136.4 ± 3.27	101.3 ± 0.1	35.1 ± 0.1	25.76 ± 0.1	0.350 ± 0.001
GMOA-G3-OH	34.91 ± 0.83	30.6 ± 0.3	4.3 ± 03	12.3 ± 0.9	0.043 ± 0.003
PF-68	11.1 ± 0.1	10.4 ± 0.1	0.7 ± 0.1	6.6 ± 1.0	0.007 ± 0.001

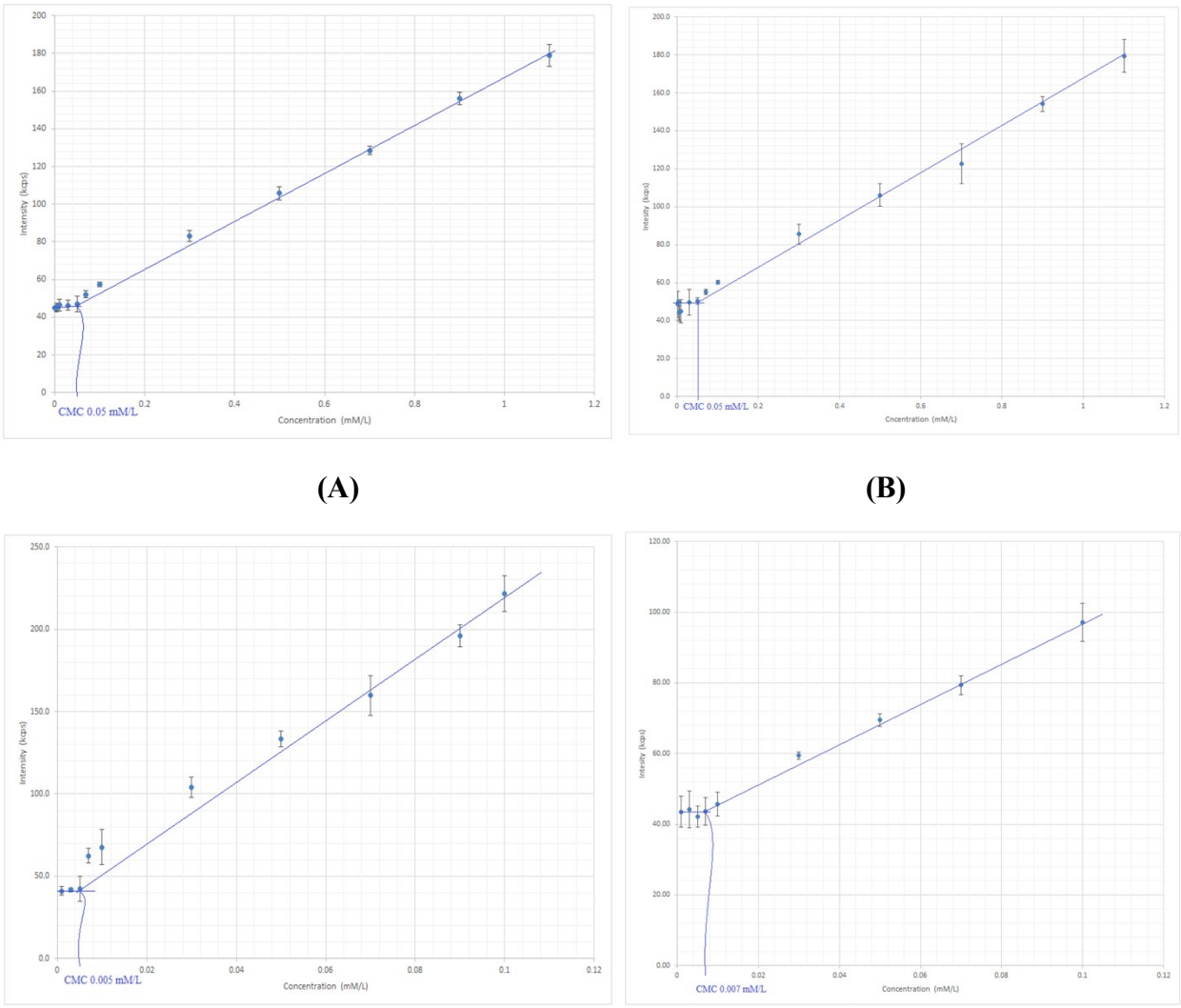


Fig. S1. CMC of (a) GMS-G2-OH, (B) GMOA-G2-OH, (C) GMS-G3-OH, (D) GMOA-G3-OH.

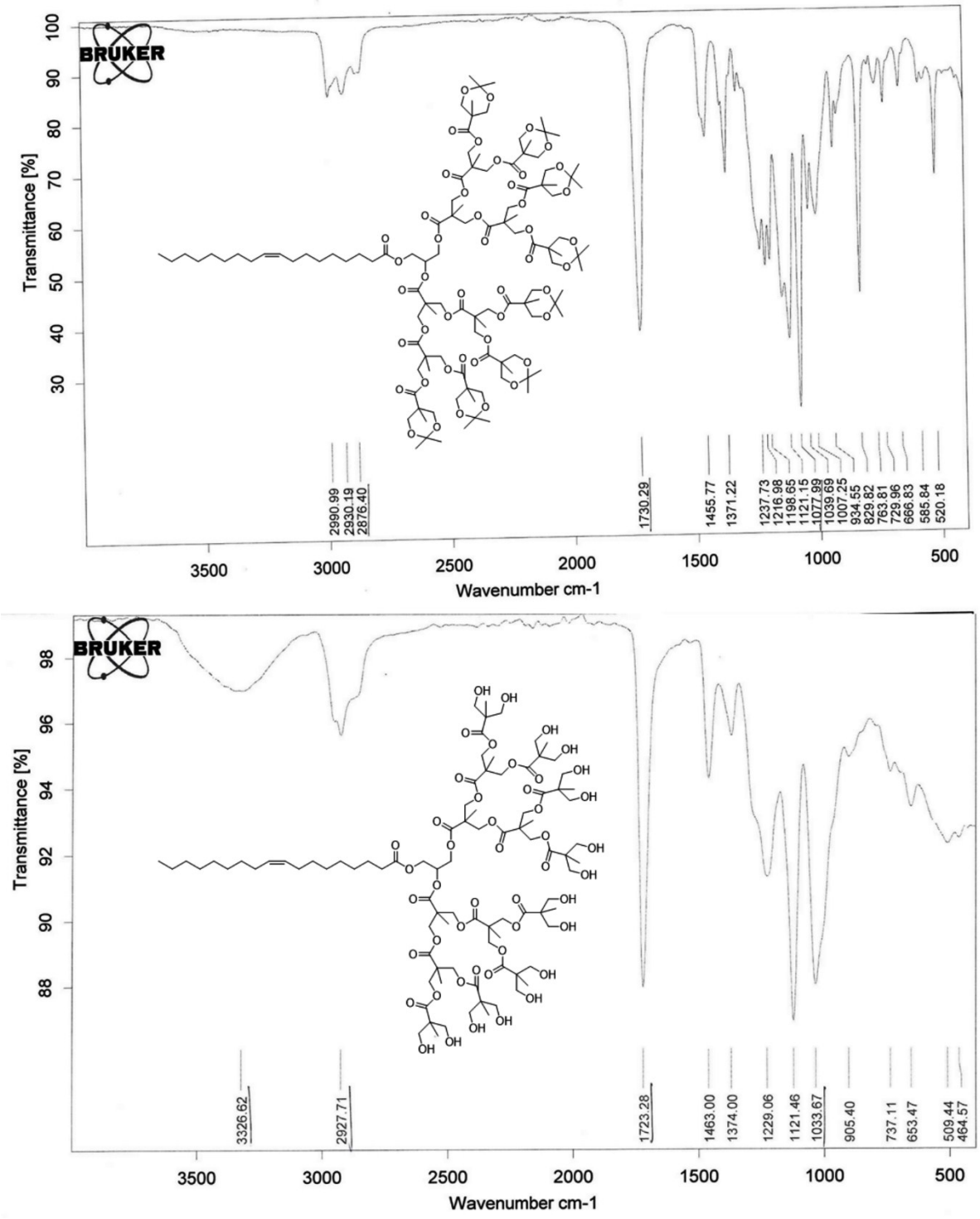
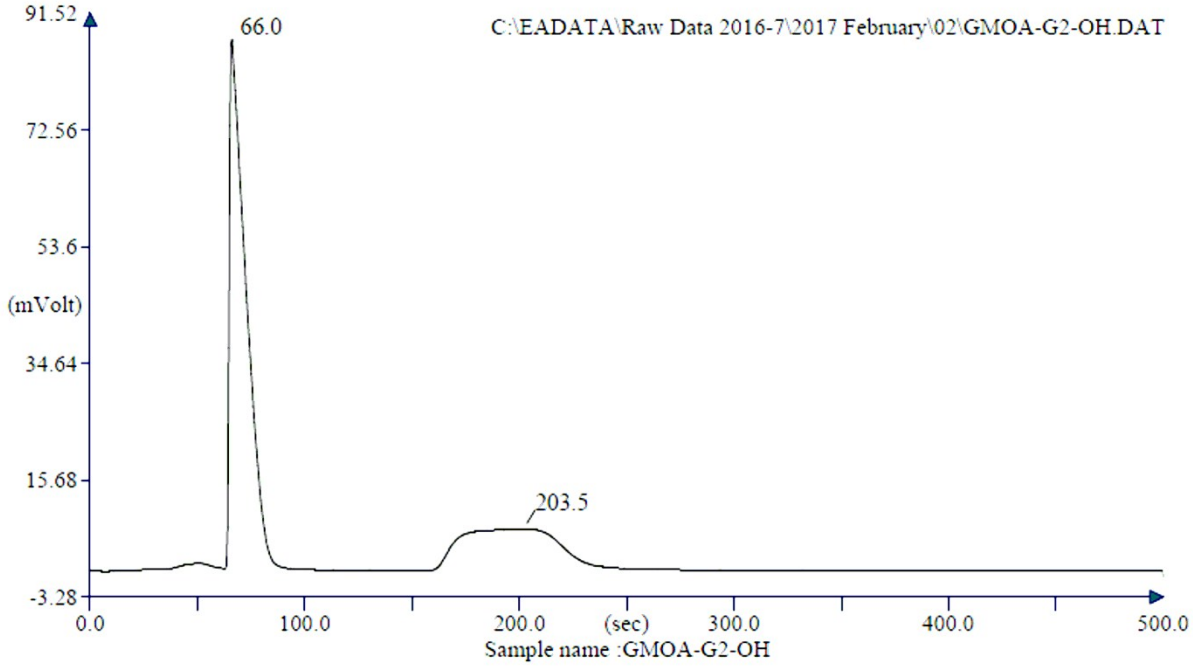
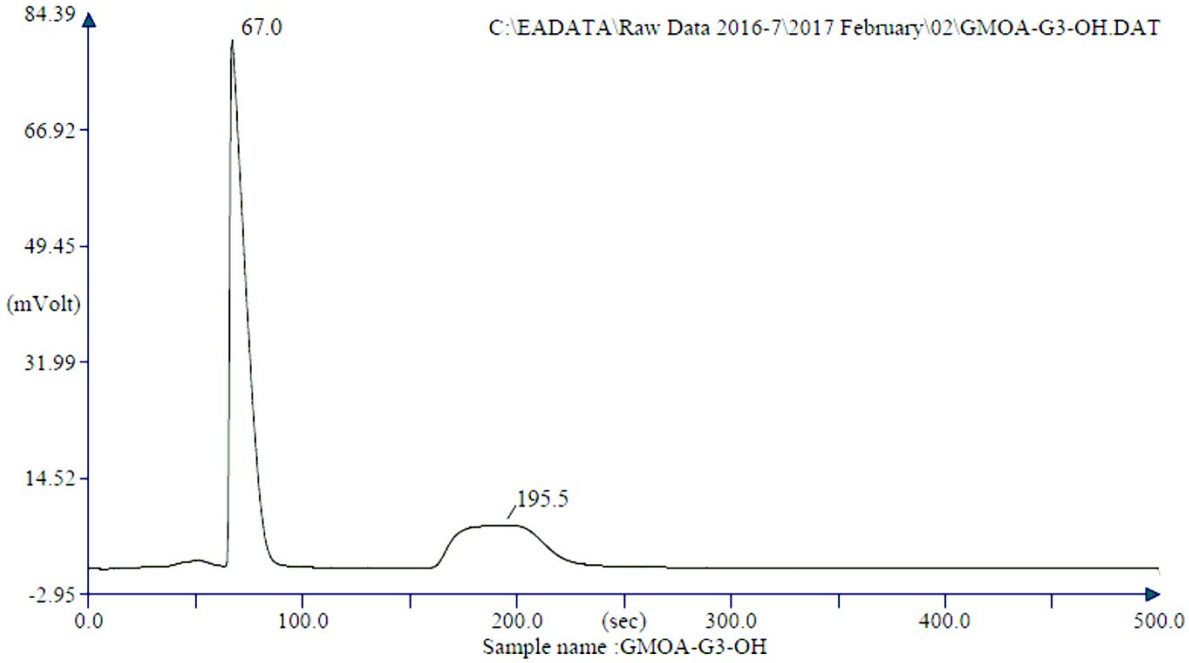


Fig. S2. Comparative FT-IR of GMOA-G3-Me and GMOA-G3-OH for confirmation of deprotection of acetonide group.



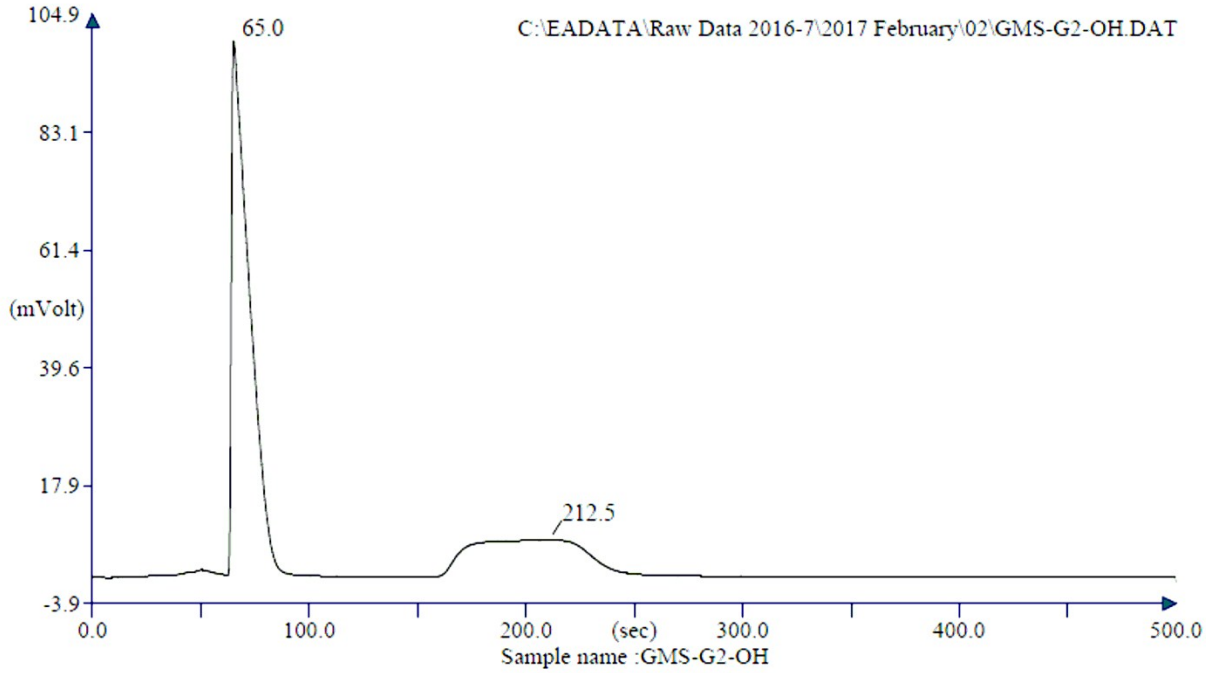
Retention Time (min)	Element Name	Element %
1.100	Carbon	55.879
3.392	Hydrogen	8.639
		64.518

Fig. S3. Elemental analysis chromatogram of GMOA-G2-OH.



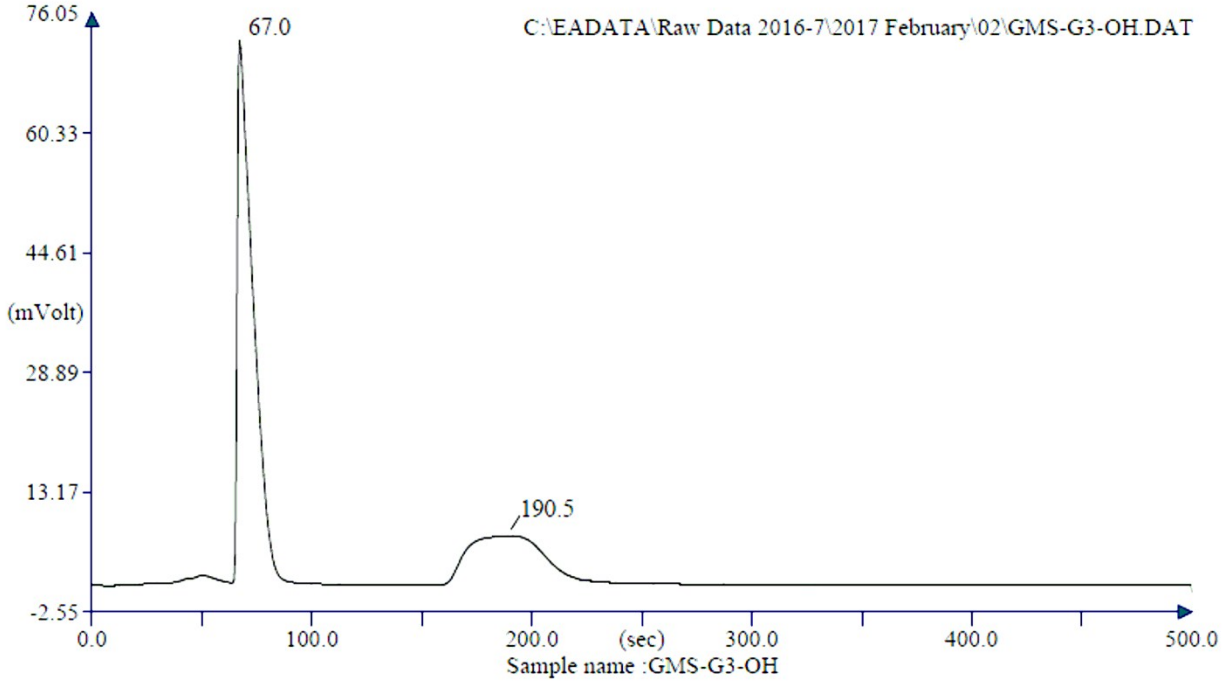
Retention Time (min)	Element Name	Element %
1.117	Carbon	54.625
3.258	Hydrogen	7.869
		62.495

Fig. S4. Elemental analysis chromatogram of GMOA-G3-OH.



Retention Time (min)	Element Name	Element %
1.083	Carbon	57.314
3.542	Hydrogen	8.623
		65.937

Fig. S5. Elemental analysis chromatogram of GMS-G2-OH.



Retention Time (min)	Element Name	Element %
1.117	Carbon	55.082
3.175	Hydrogen	8.063
		63.145

Fig. S6. Elemental analysis chromatogram of GMS-G3-OH.

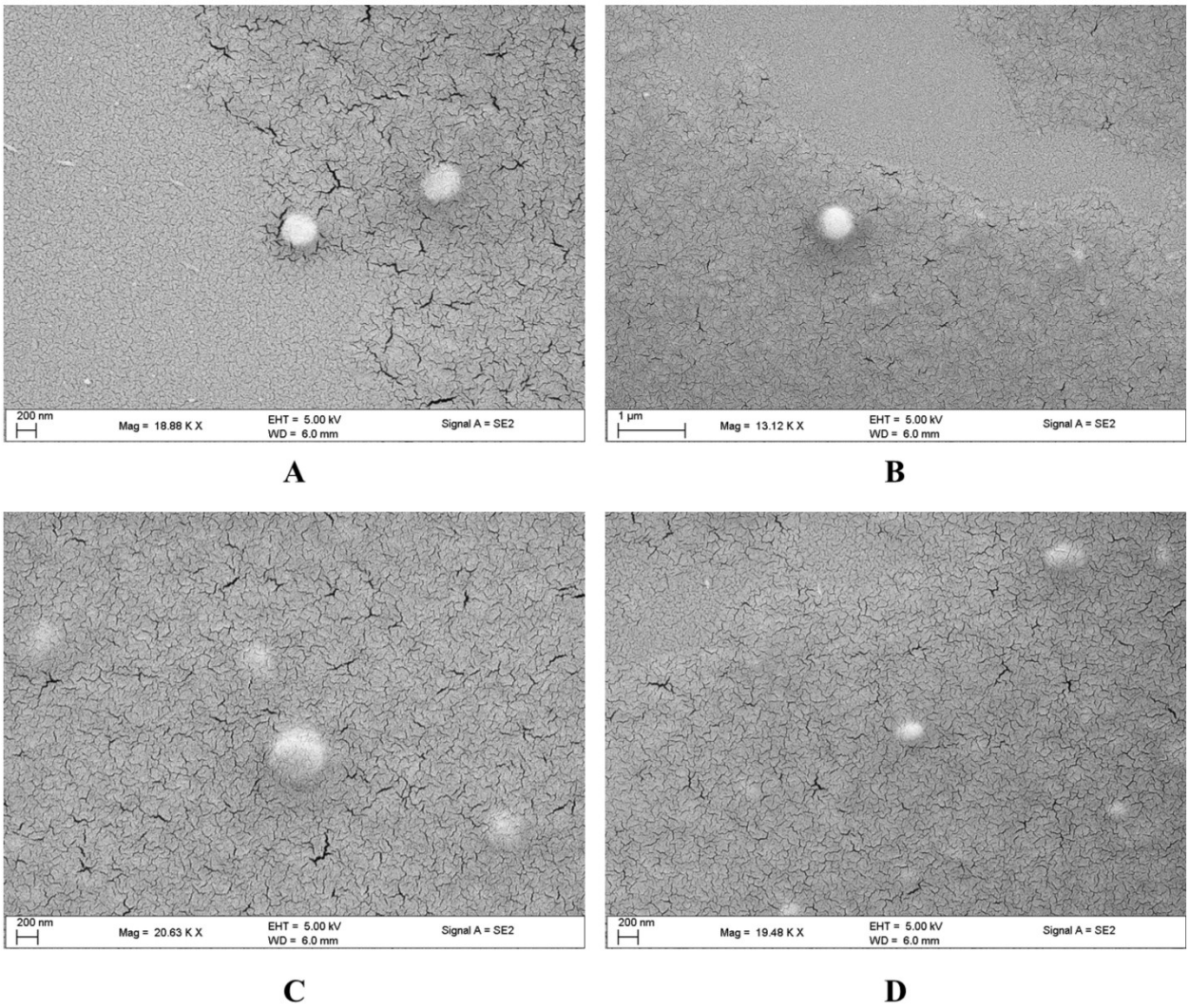


Fig. S7. SEM images showing morphology of SLNs stabilized using A) GMOA-G2-OH, B) GMS-G2-OH, C) GMOA-G3-OH and D) GMS-G3-OH ADs.

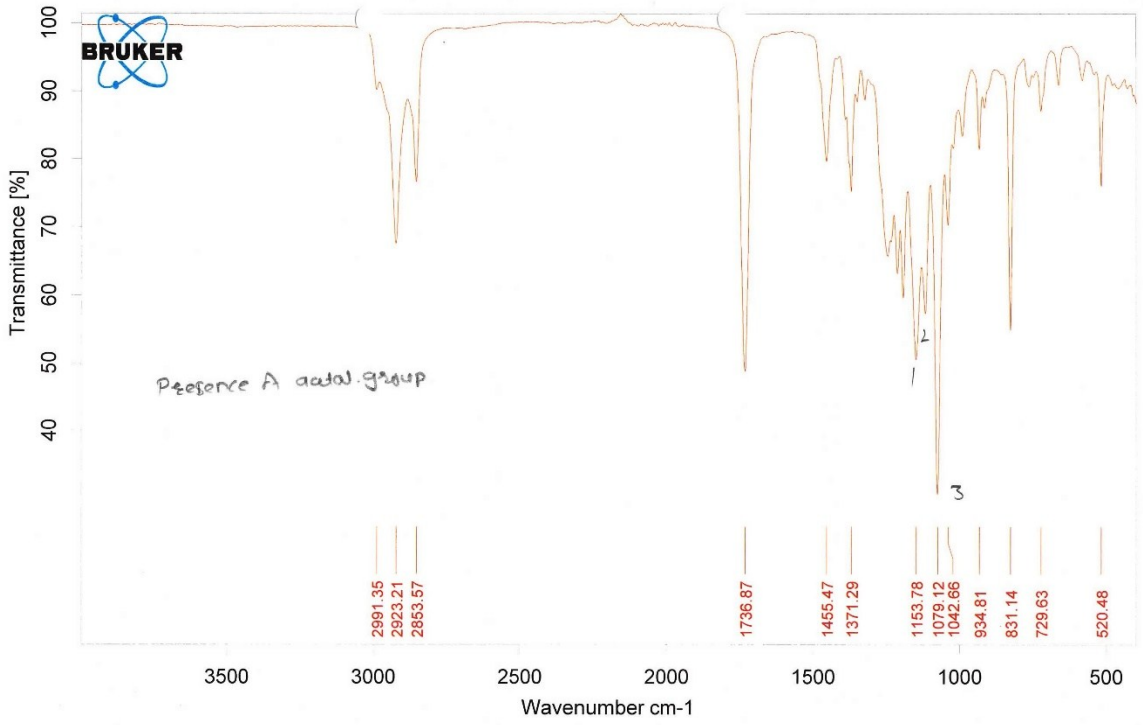


Fig. S8. FT-IR spectra of GMS-G1-Me.

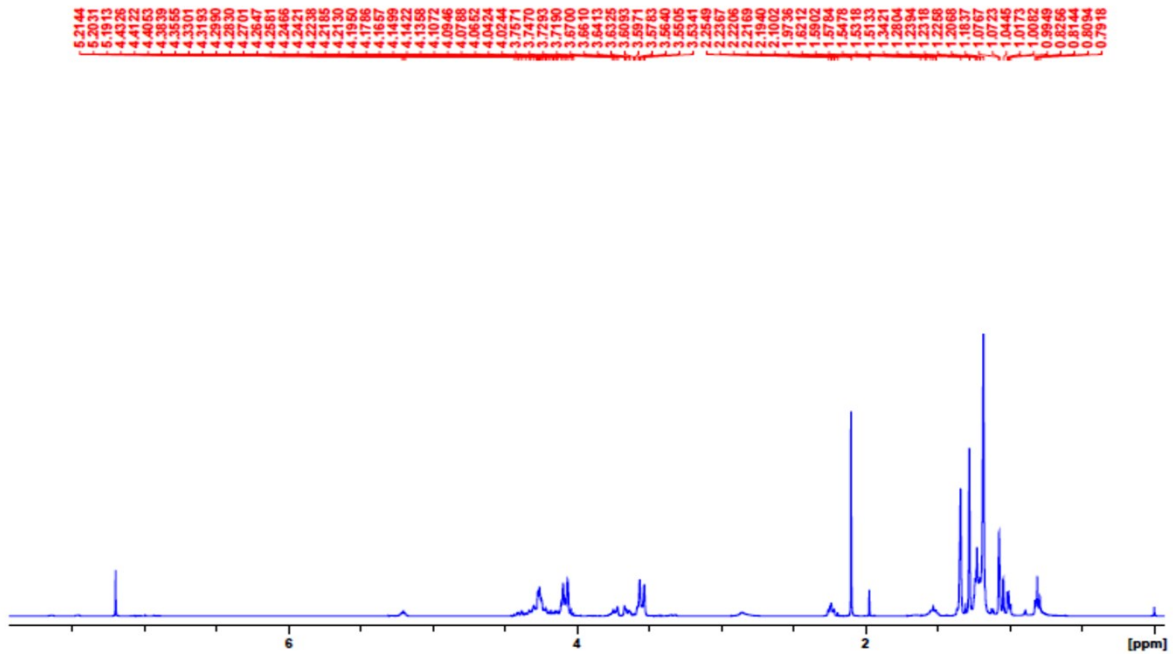


Fig. S9. ^{1}H NMR of GMS-G1-Me.

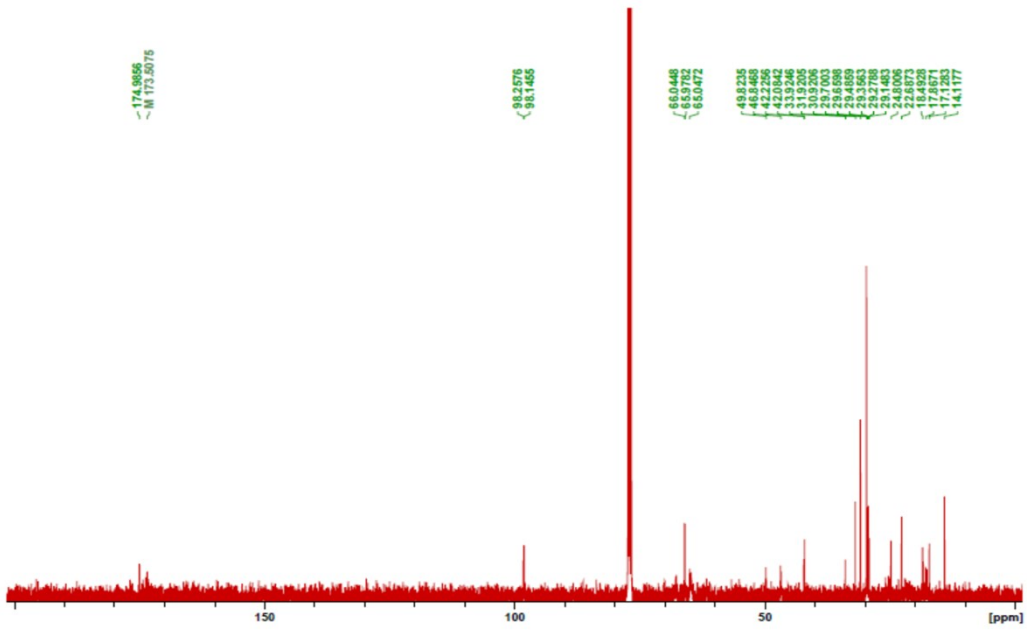
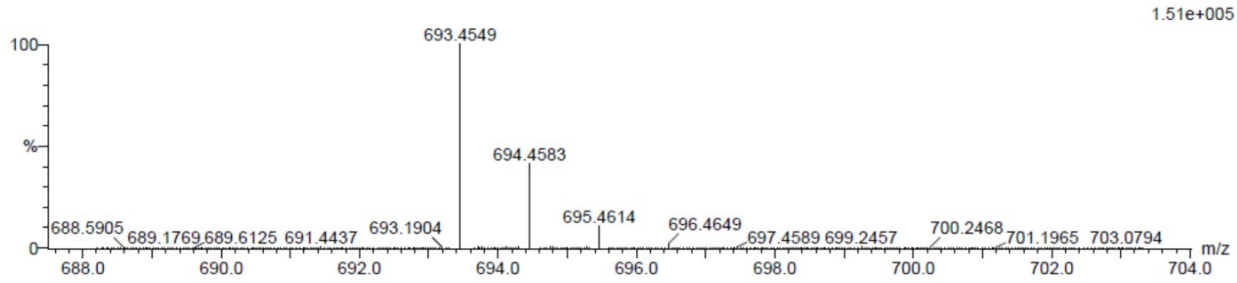


Fig. S10. ^{13}C NMR of GMS-G1-OH.



Minimum:				-1.5			
Maximum:		5.0	4.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
693.4549	693.4554	-0.5	-0.7	4.5	468.7	0.0	C37 H66 O10 Na

Fig. S11. HRMS of GMS-G1-Me.

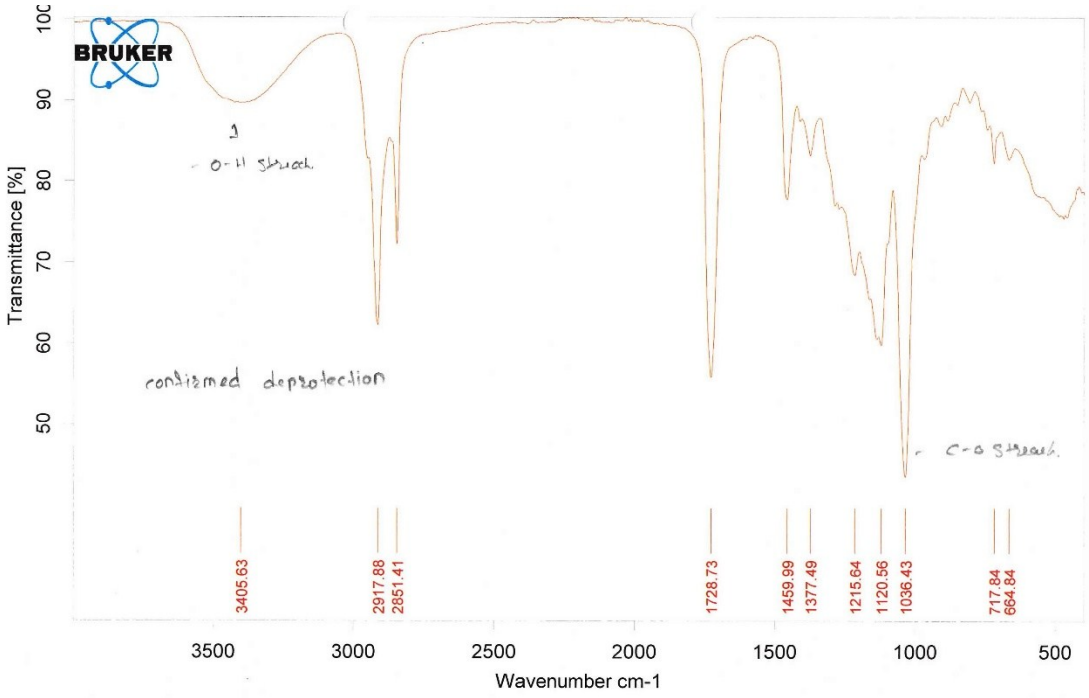


Fig. S12. FT-IR spectra of GMS-G1-OH.

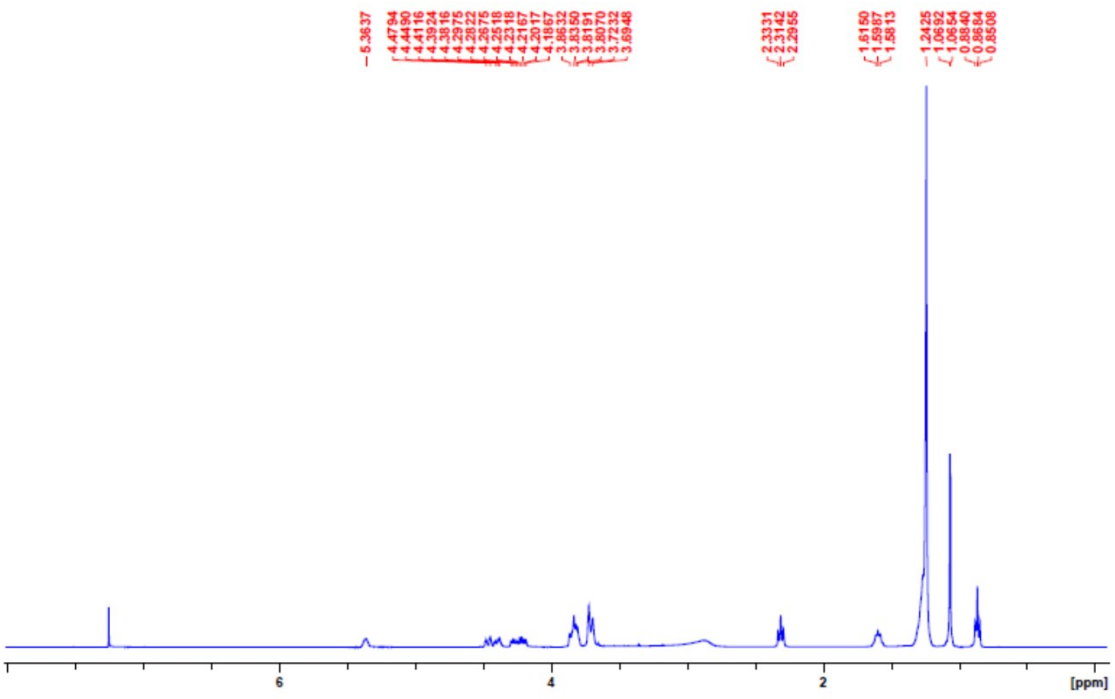


Fig. S13. ^{1}H NMR of GMS-G1-OH.

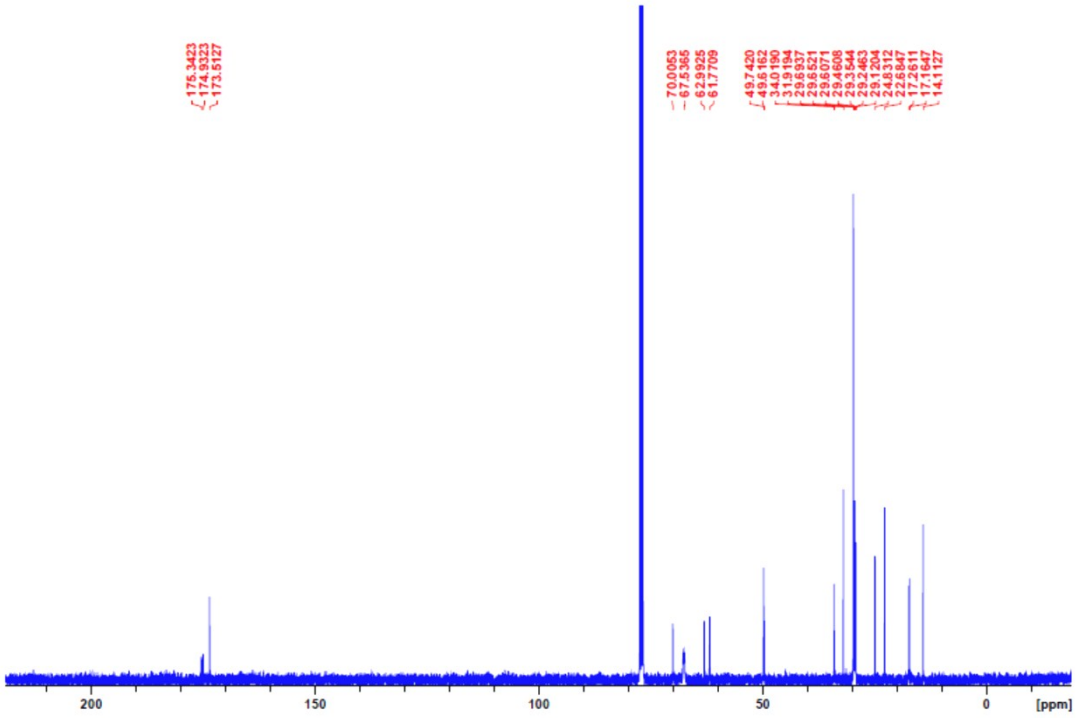
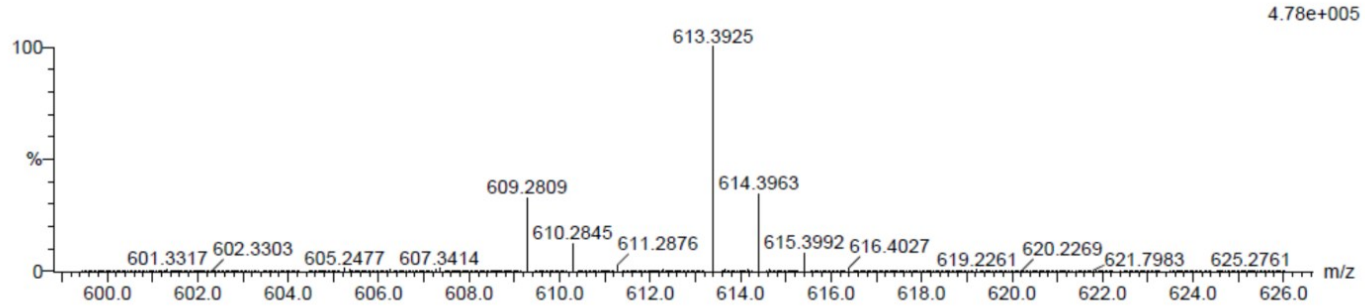


Fig. S14. ^{13}C NMR of GMS-G1-OH.



Minimum:				-1.5			
Maximum:		5.0	4.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
613.3925	613.3928	-0.3	-0.5	2.5	538.3	0.0	C31 H58 O10 Na

Fig. S15. HRMS of GMS-G1-OH.

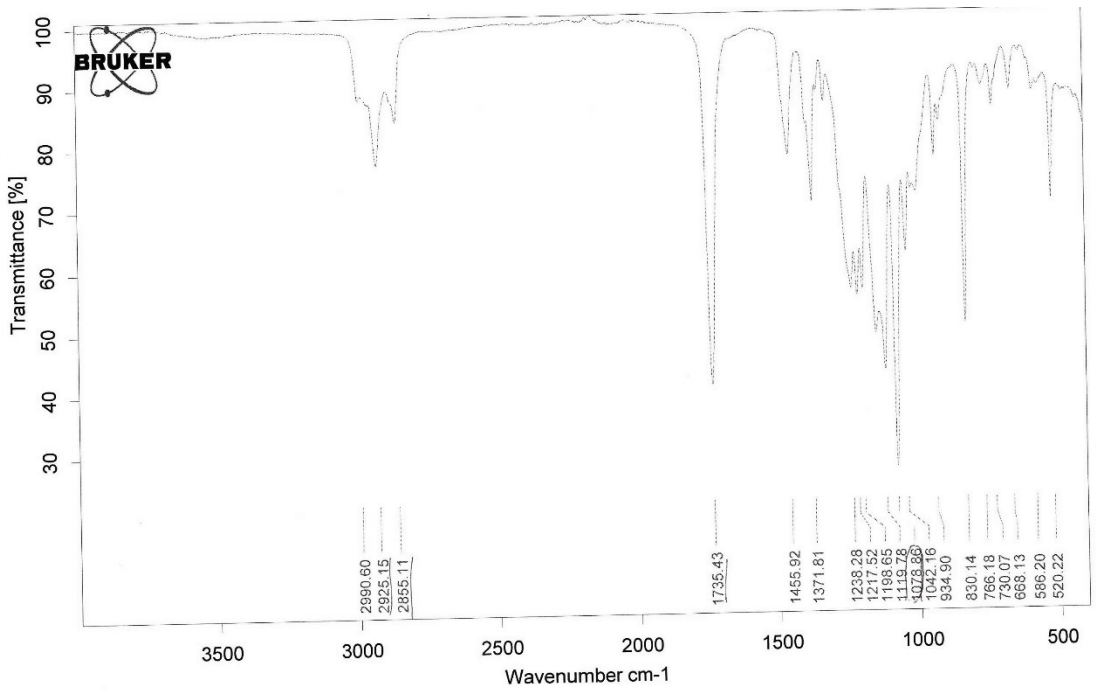


Fig. S16. FT-IR spectra of GMS-G2-Me.

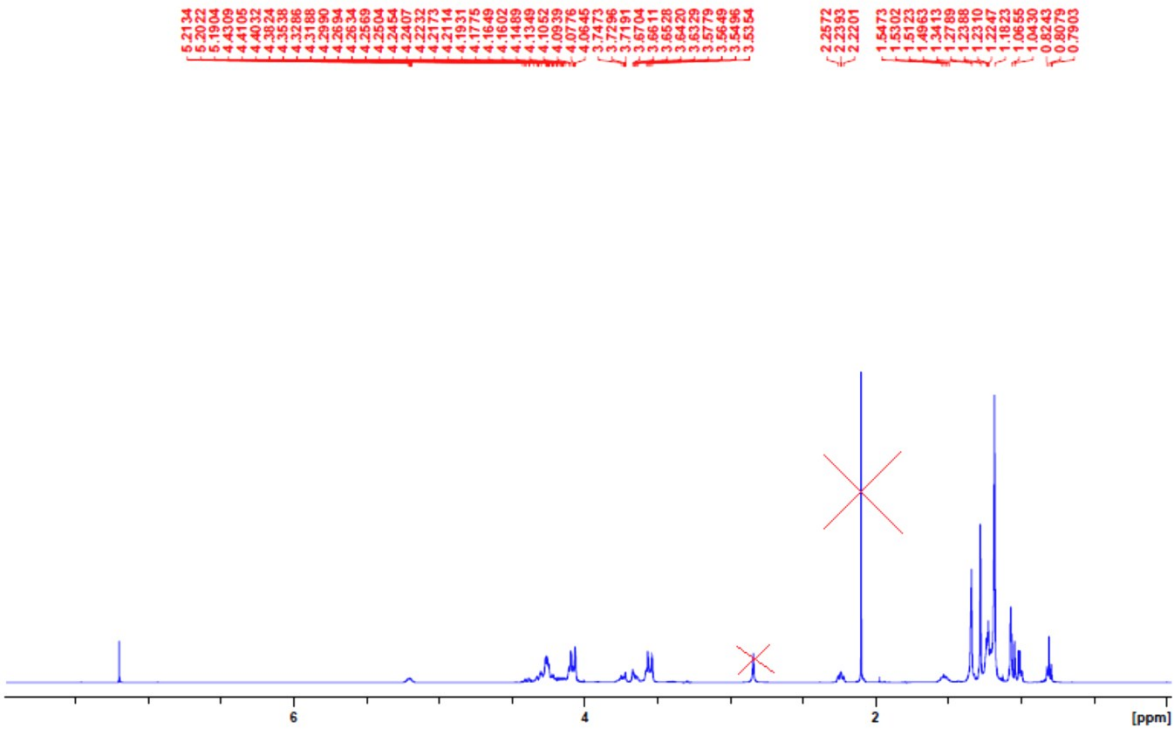


Fig. S17. ^{1}H NMR of GMS-G2-Me.

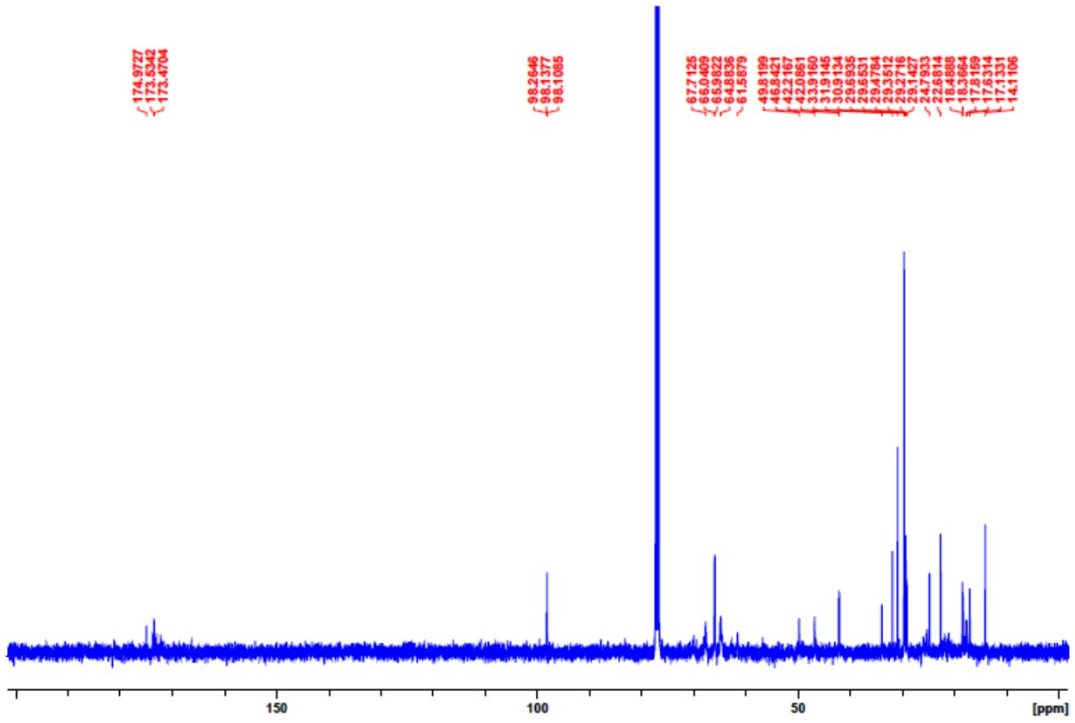
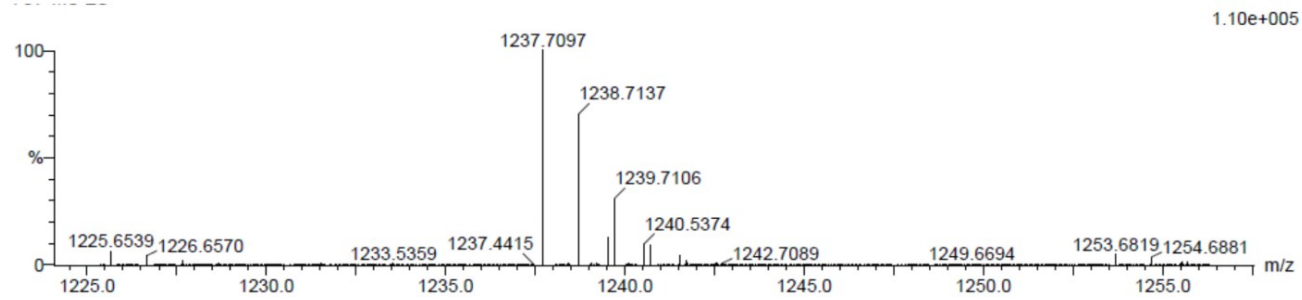


Fig. S18. ^{13}C NMR of GMS-G2-Me.



Minimum:				-1.5			
Maximum:		5.0	4.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1237.7097	1237.7073	2.4	1.9	10.5	243.3	0.0	C63 H106 O22 Na

Fig. S19. HRMS of GMS-G2-Me.

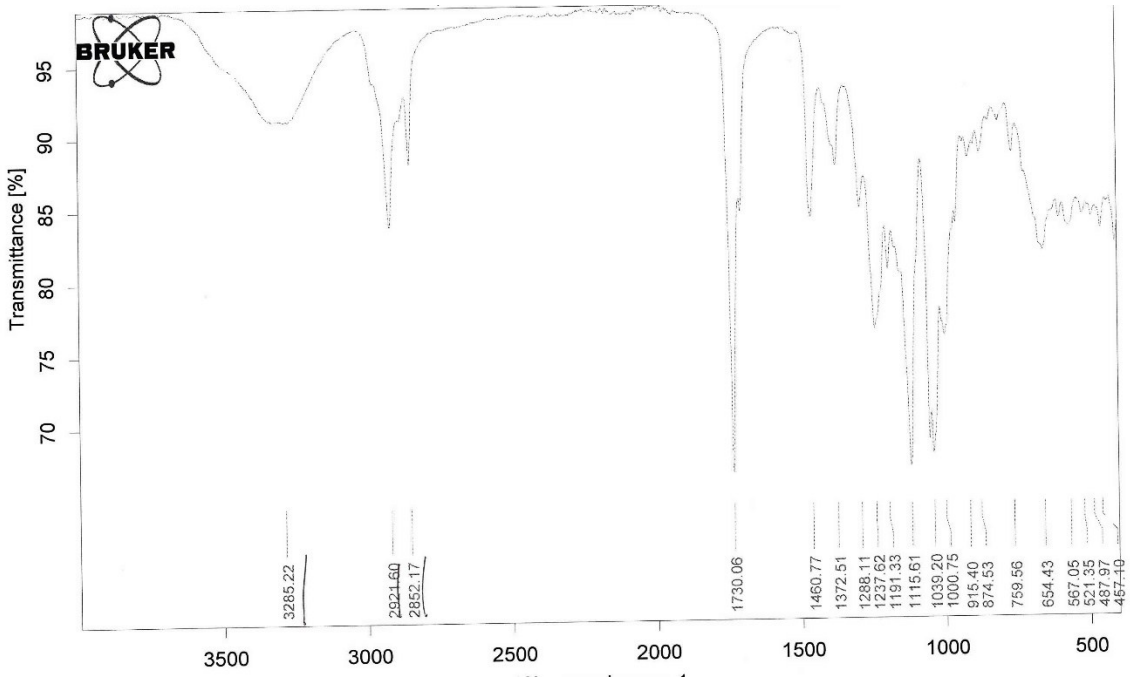


Fig. S20. FT-IR spectra of GMS-G2-OH.

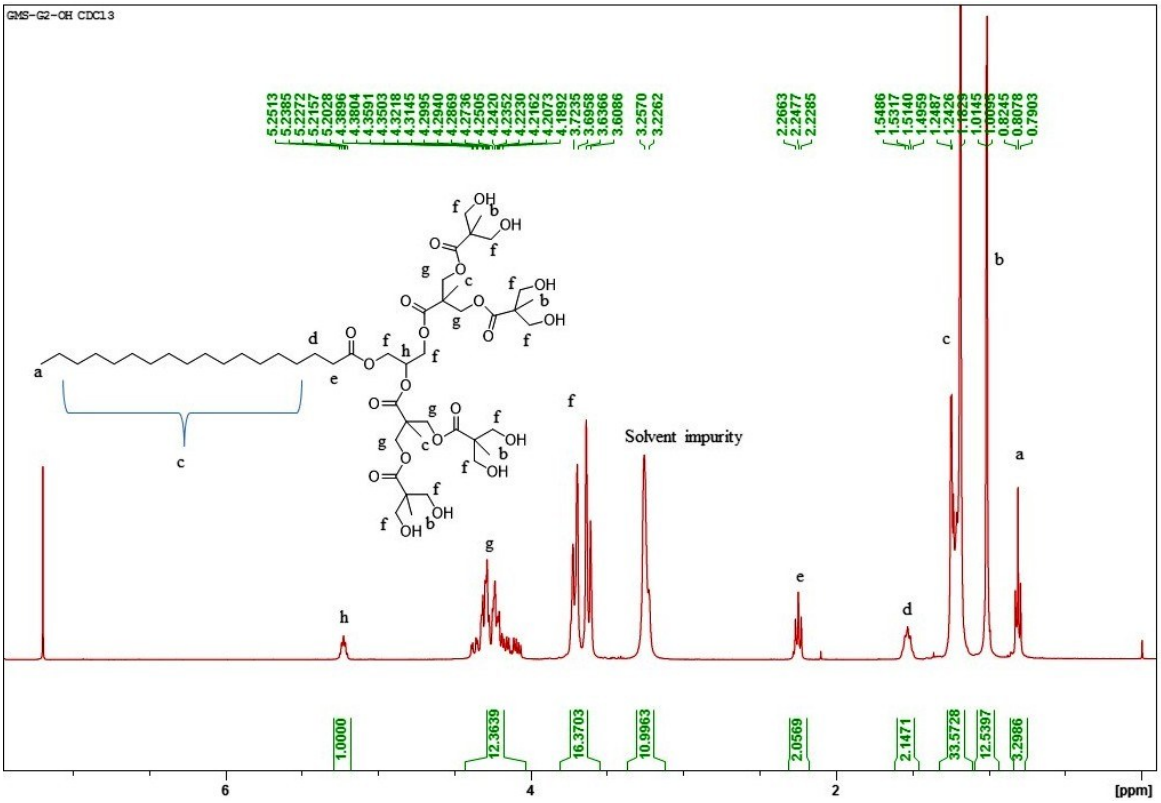


Fig. S21. ^{1}H NMR of GMS-G2-OH.

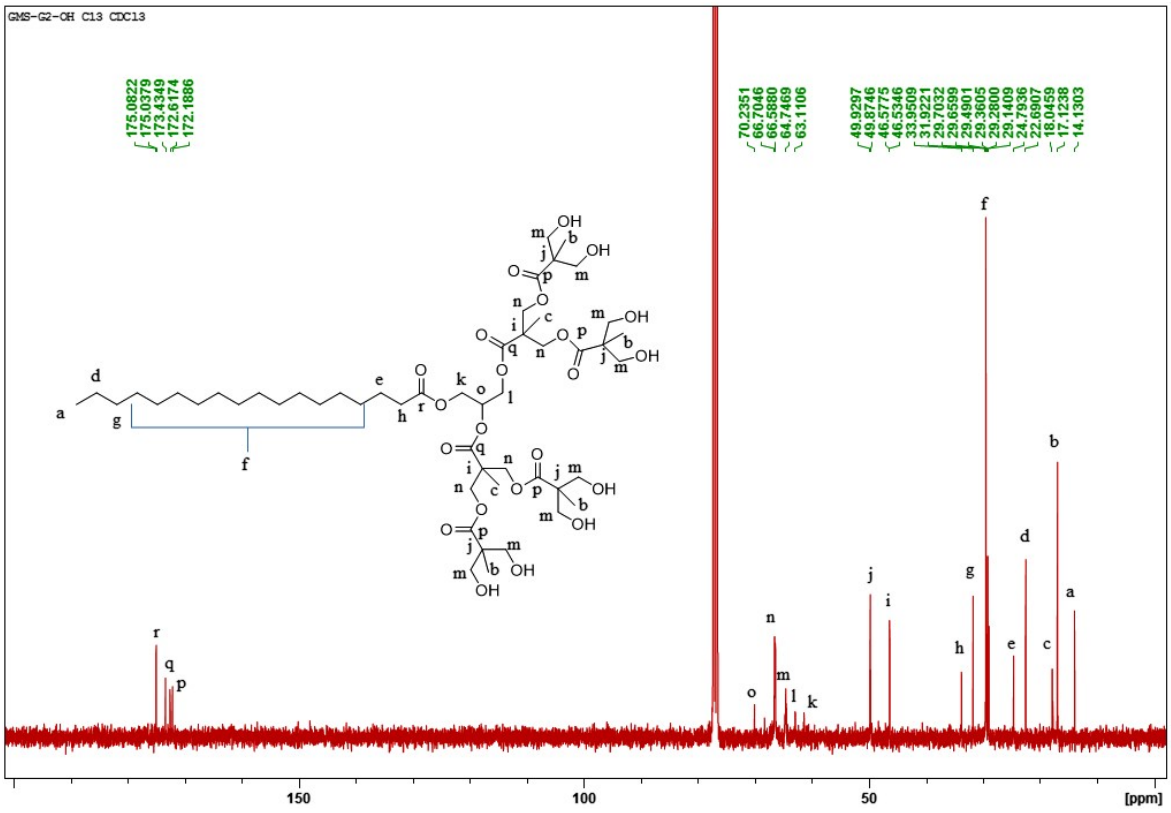
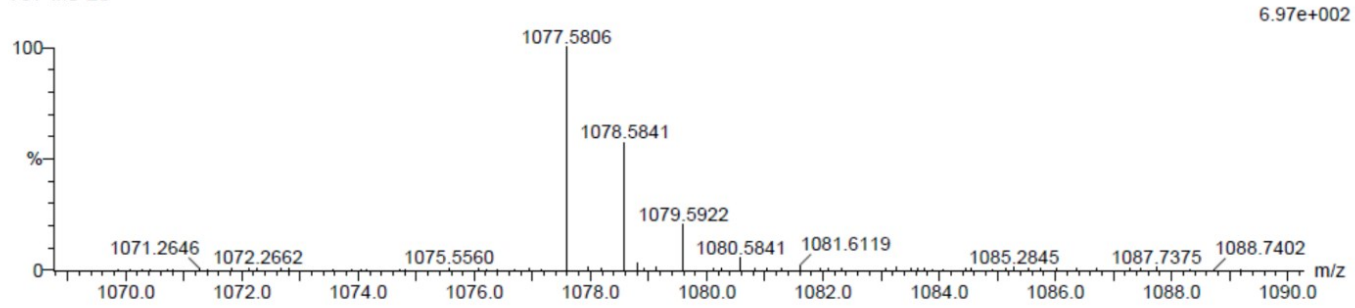


Fig. S22. ^{13}C NMR of GMS-G2-OH.



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1077.5806	1077.5821	-1.5	-1.4	6.5	30.6	0.0	C51 H90 O22 Na

Fig. S23. HRMS of GMS-G2-OH.

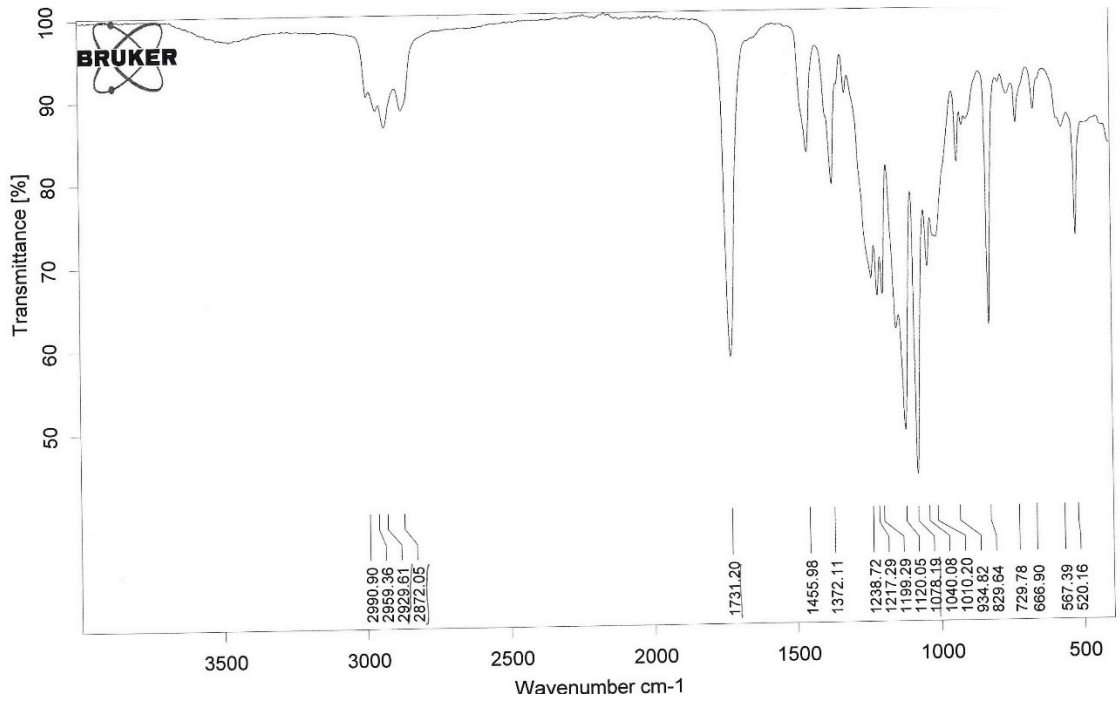


Fig. S24. FT-IR spectra of GMS-G3-Me.

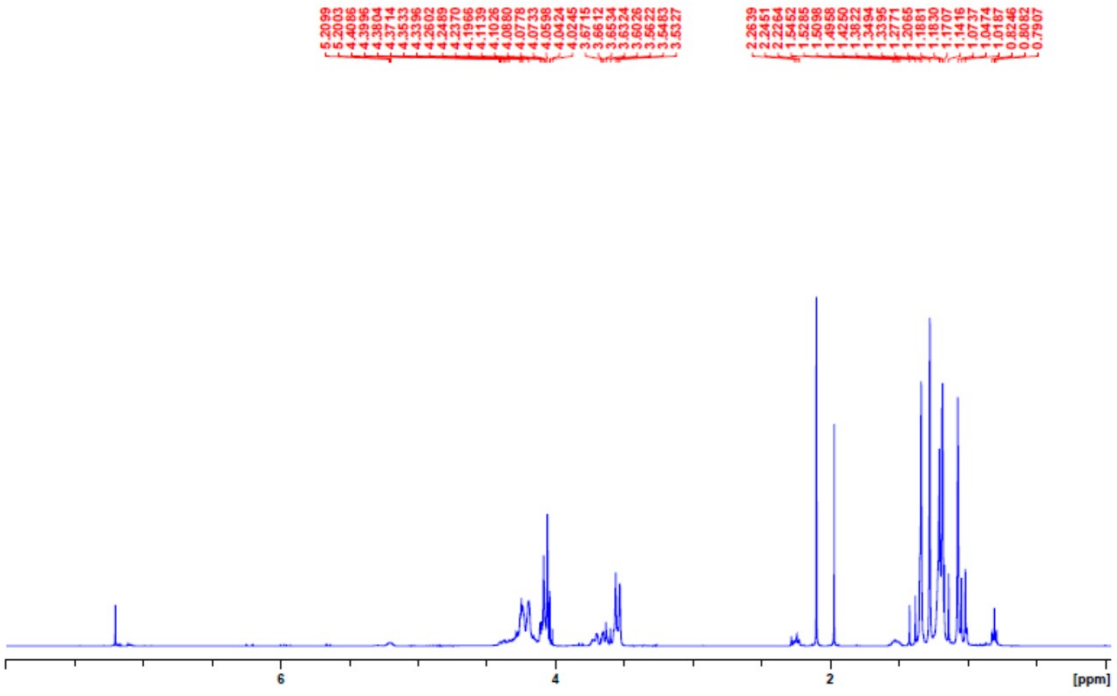


Fig. S25. ^{1}H NMR of GMS-G3-Me.

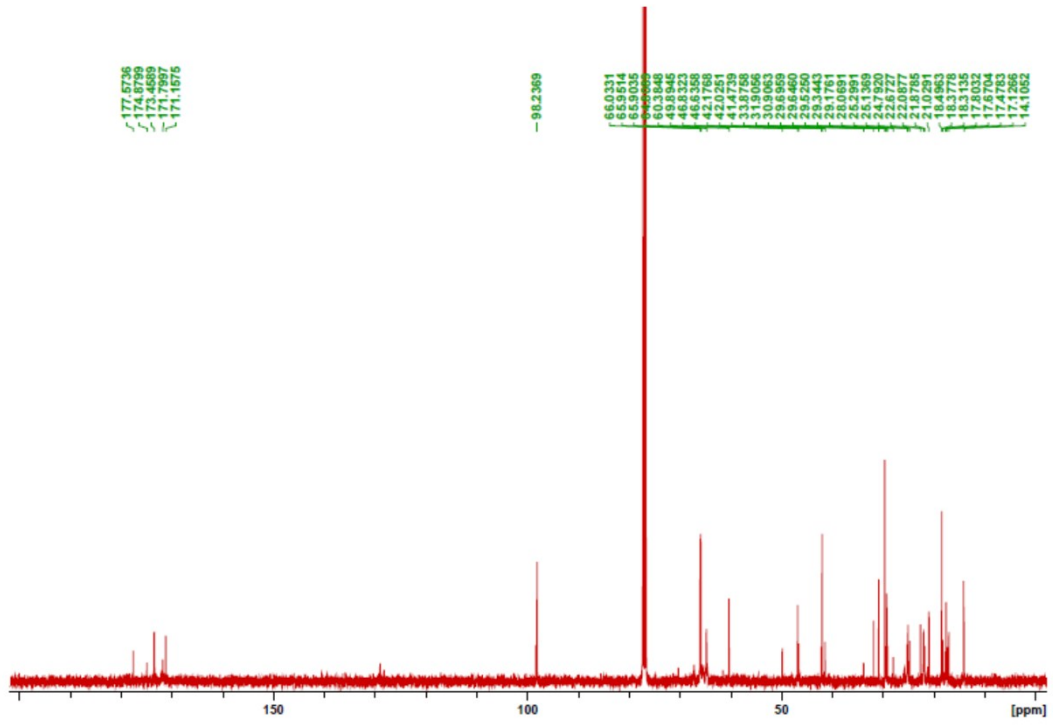
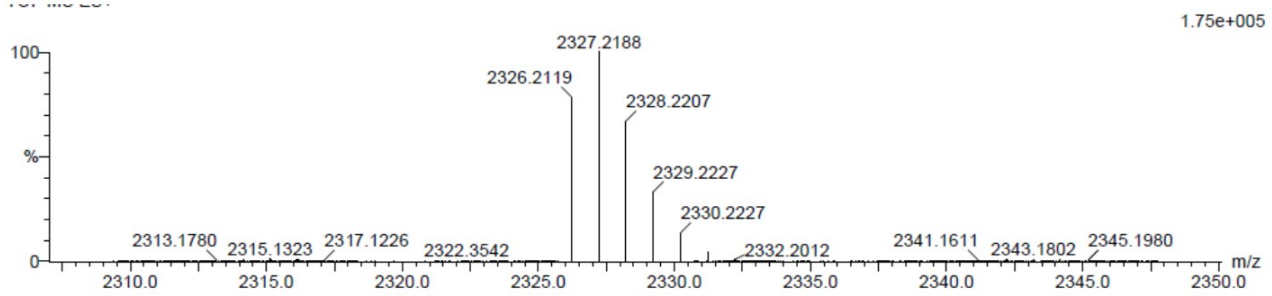


Fig. S26. ^{13}C NMR of GMS-G3-Me.



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
2326.2119	2326.2113	0.6	0.3	22.5	26.1	0.0	C115 H186 O46 Na

Fig. S27. HRMS of GMS-G3-Me.

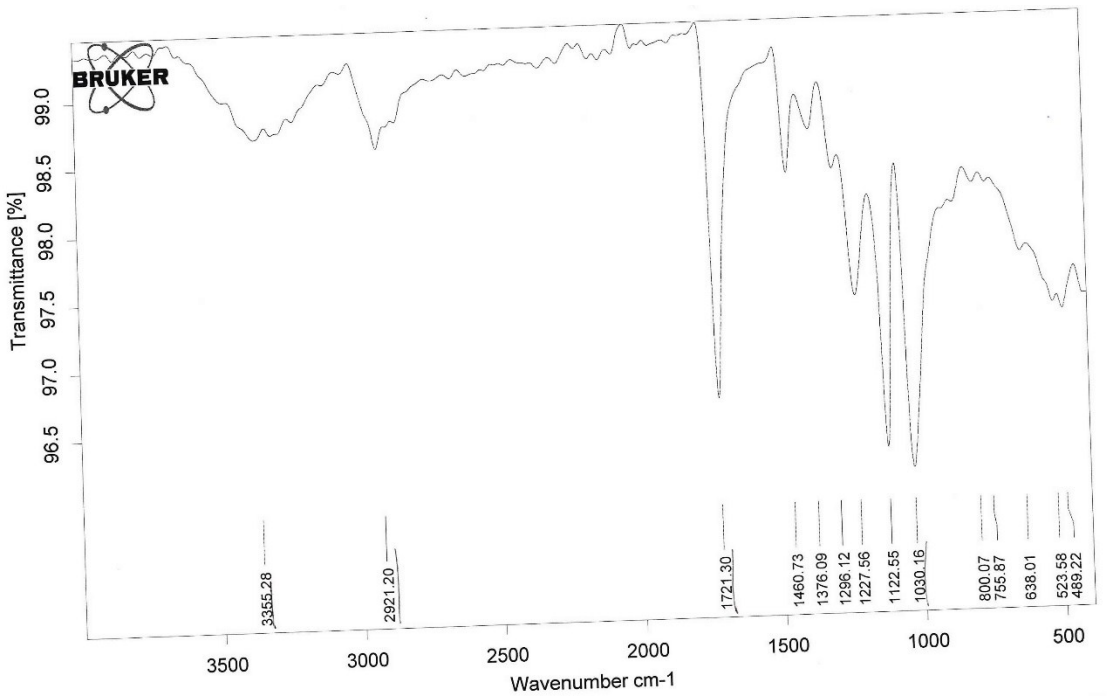


Fig. S28. FT-IR spectra of GMS-G3-Me.

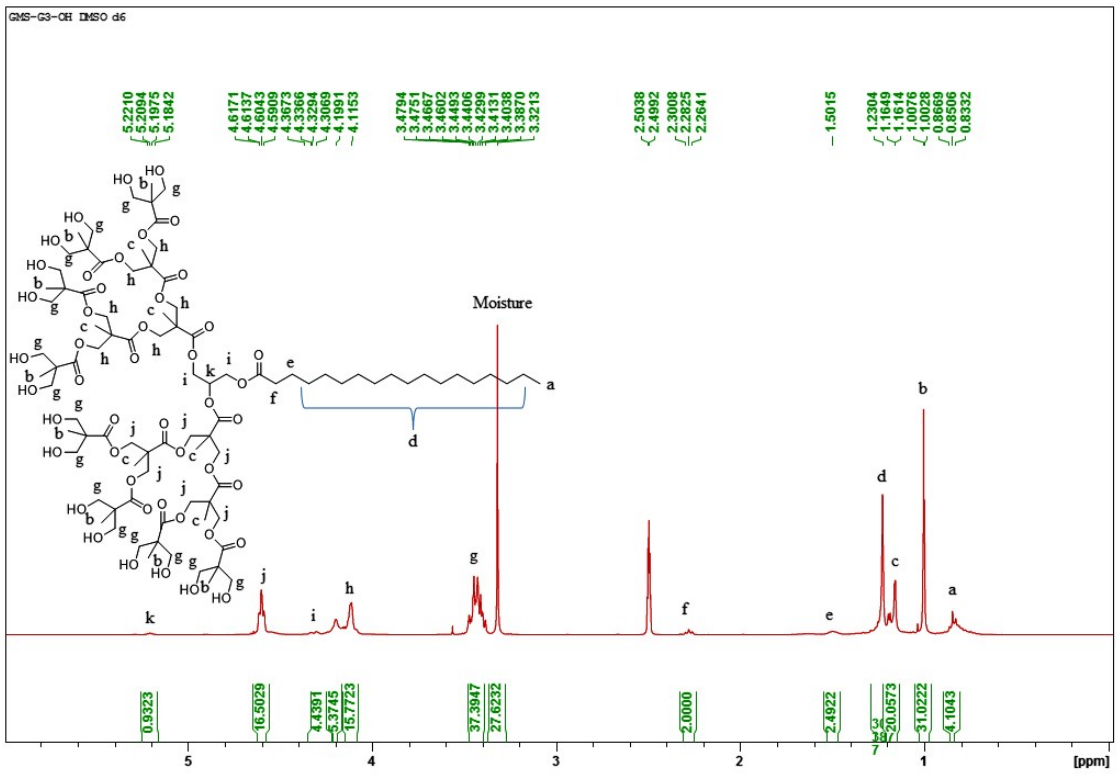


Fig. S29. ^{1}H NMR of GMS-G3-OH.

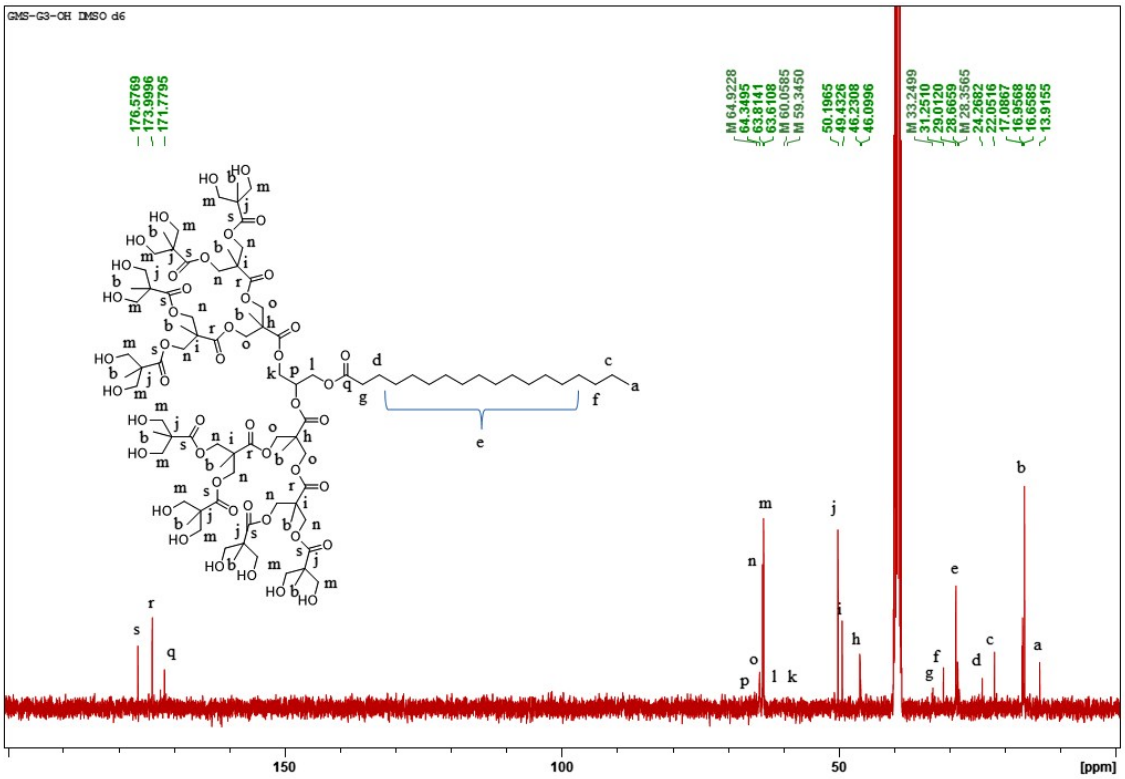
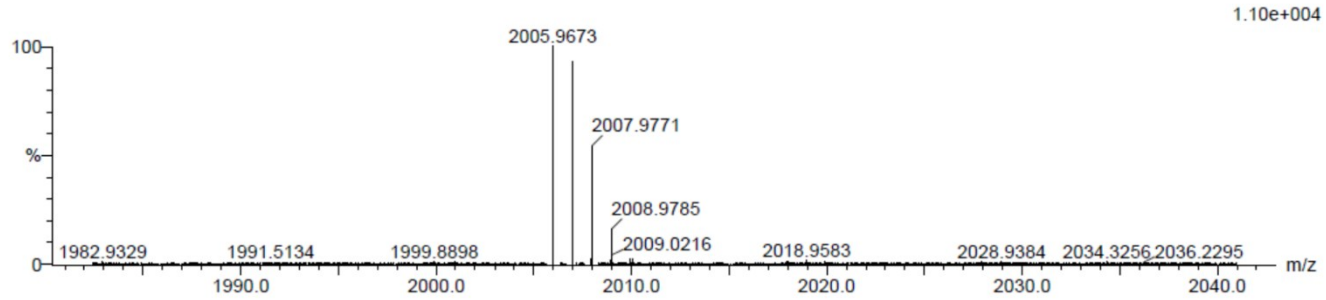


Fig. S30. ^{13}C NMR of GMS-G3-OH.



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
2005.9673	2005.9609	6.4	3.2	14.5	173.7	0.0	C91 H154 O46 Na

Fig. S31. HRMS of GMS-G3-OH.

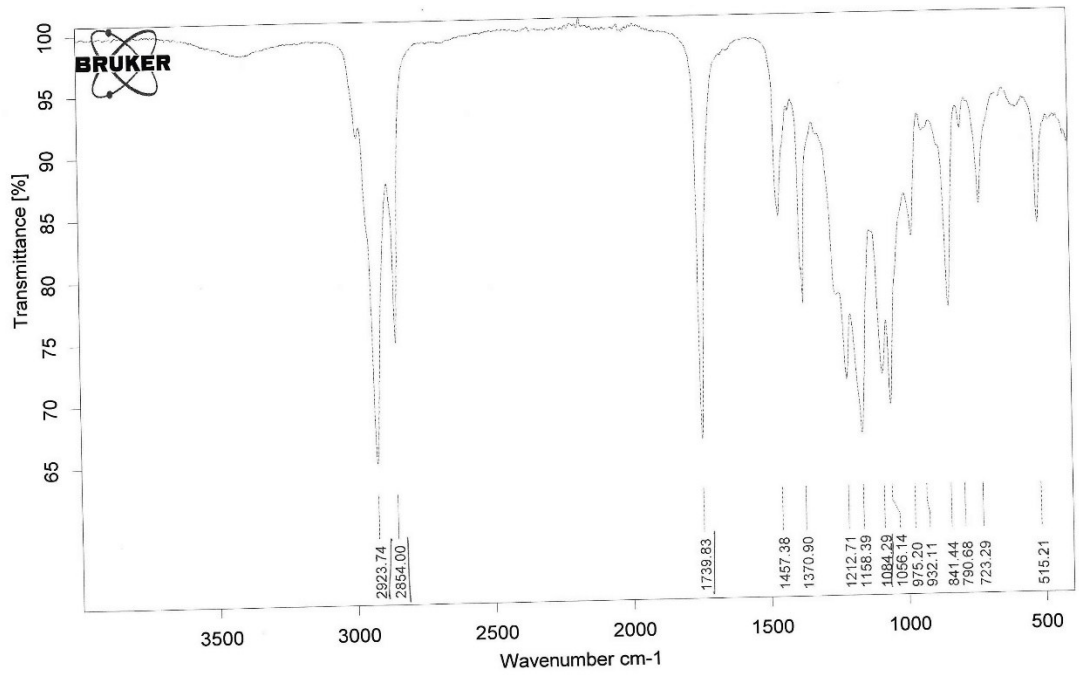


Fig. S32. FT-IR spectra of GMOA-Me.

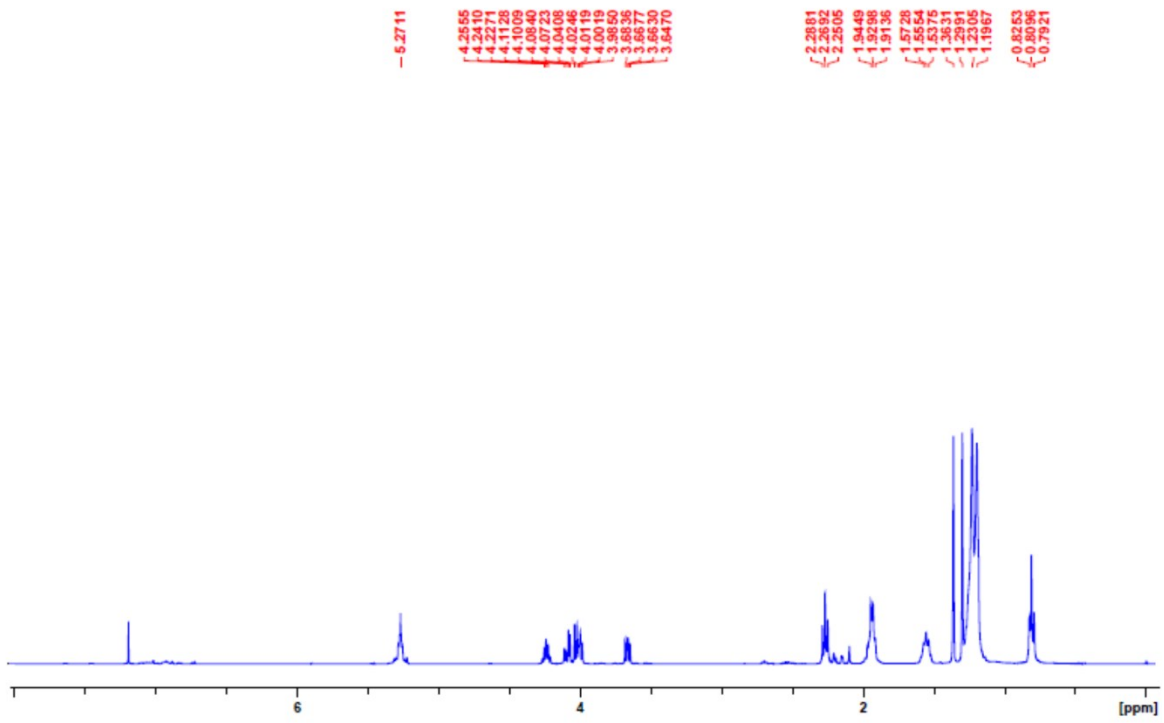


Fig. S33. ^{1}H NMR of GMOA-Me.

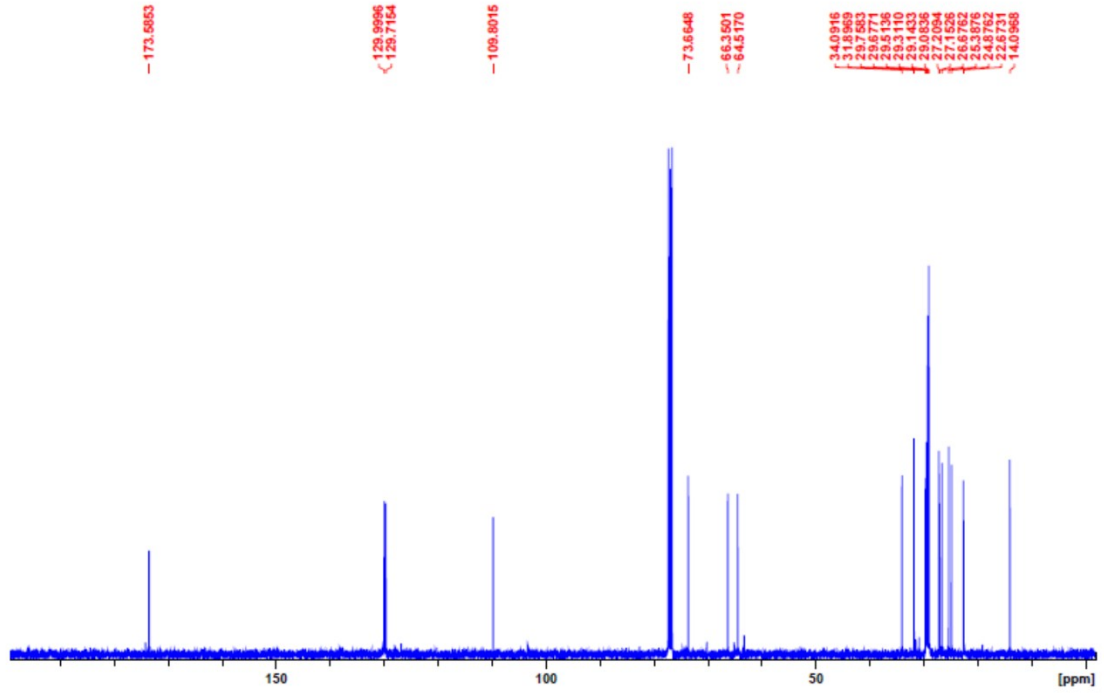


Fig. S34. ^{13}C NMR of GMOA-Me.

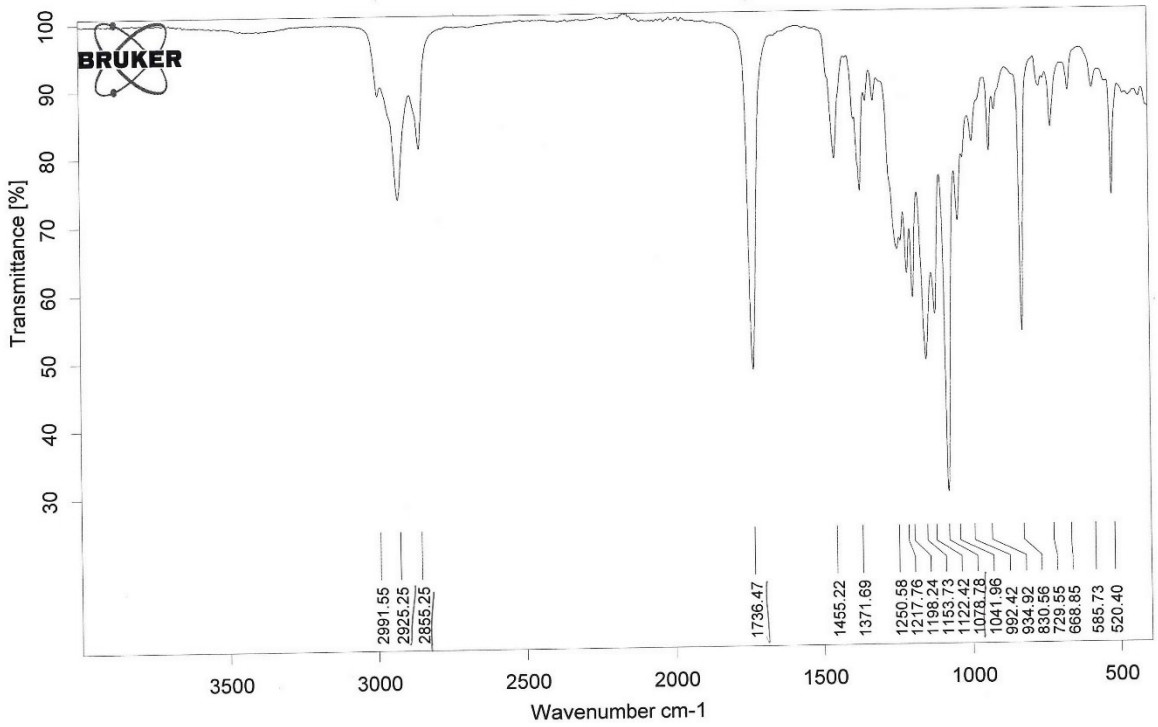


Fig. S35. FT-IR spectra of GMOA.

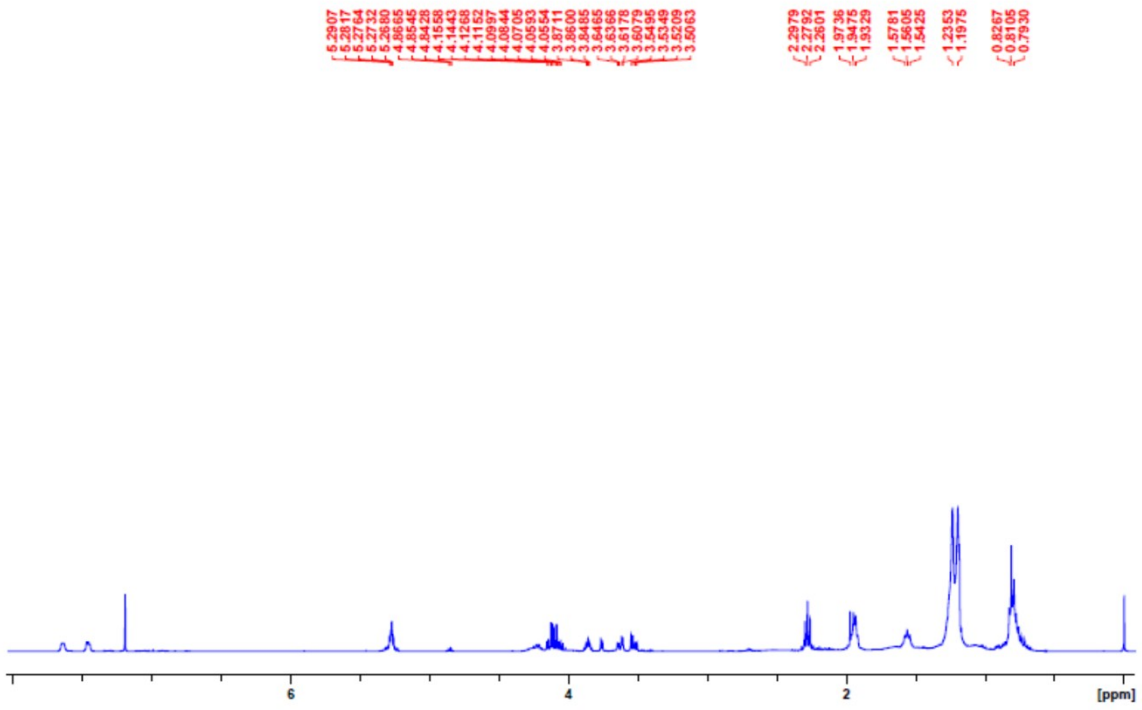


Fig. S36. ^{1}H NMR of GMOA.

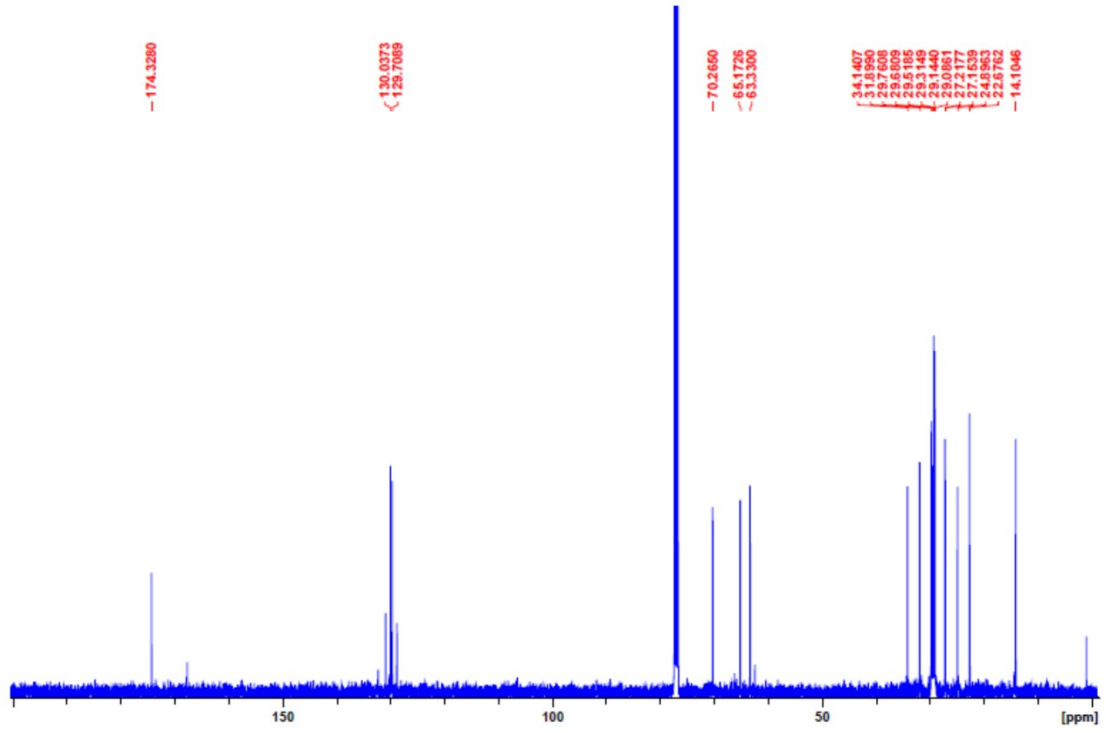


Fig. S37. ^{13}C NMR of GMOA-OH.

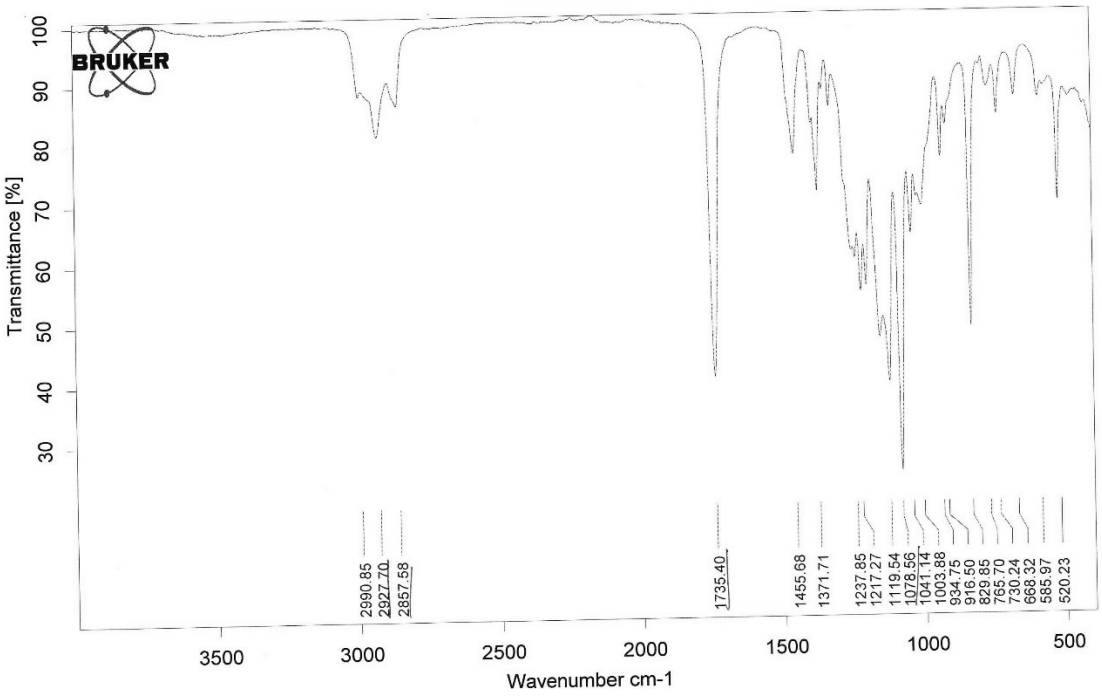


Fig. S38. FT-IR spectra of GMOA-G1-Me.

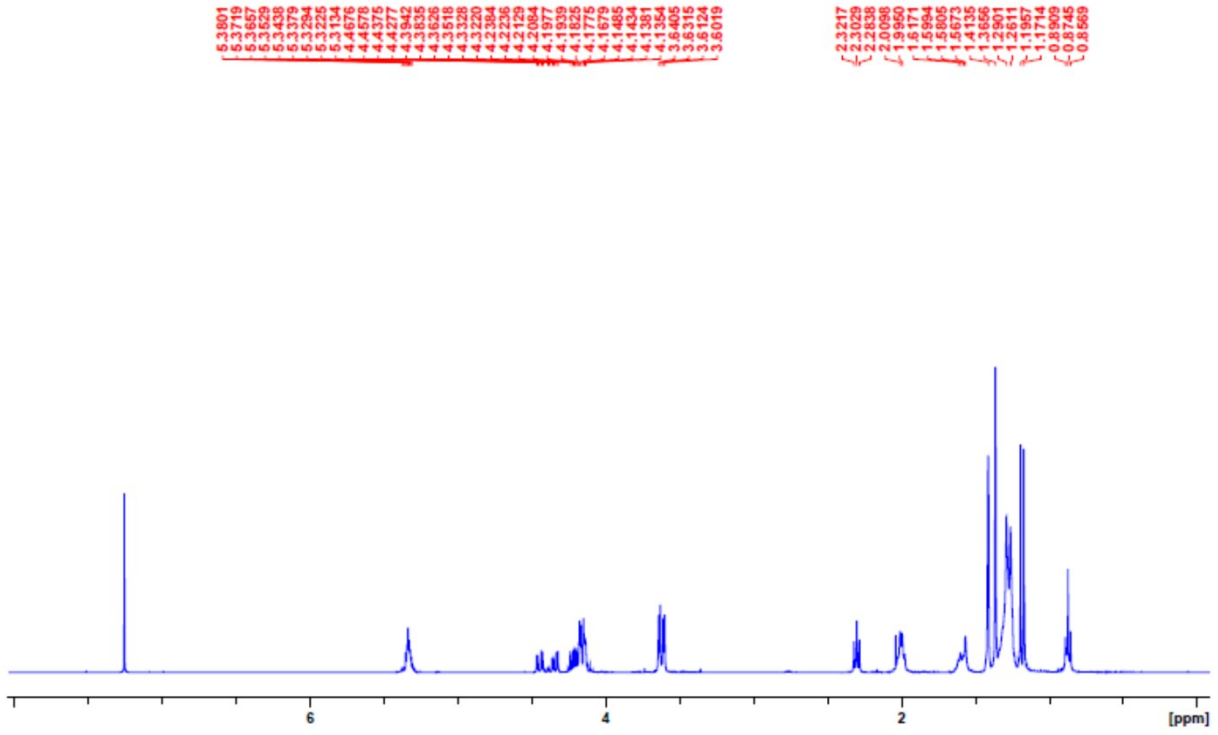


Fig. S39. ^{1}H NMR of GMOA-G1-Me.

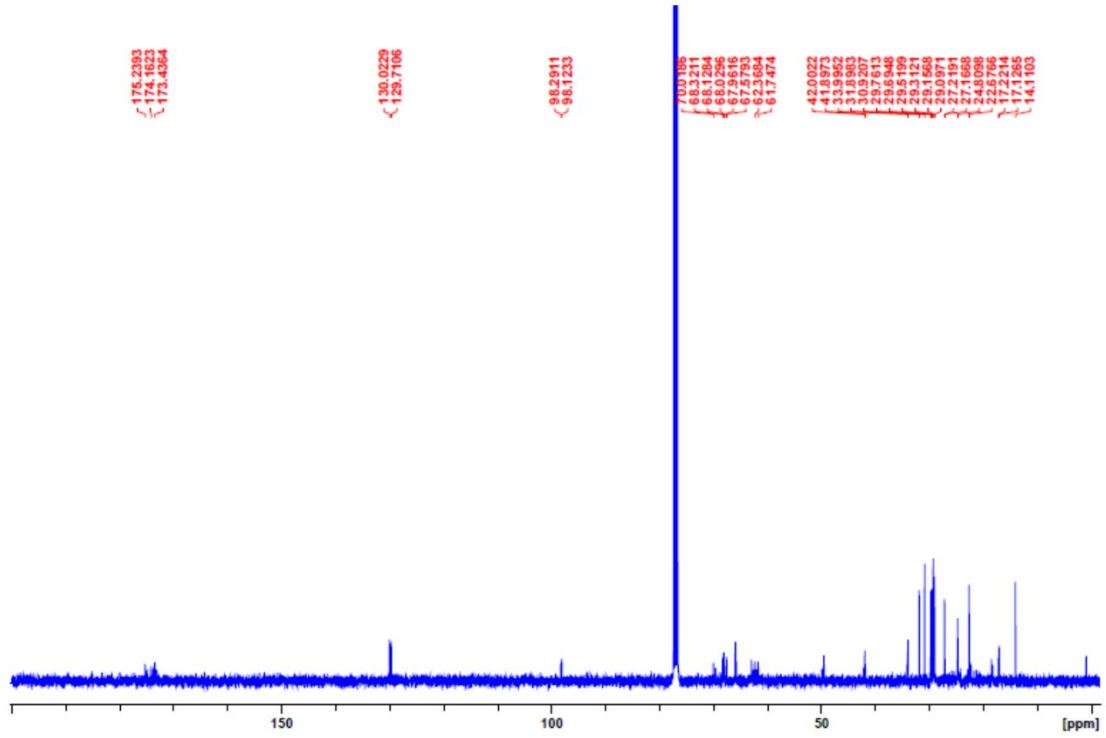


Fig. S40. ^{13}C NMR of GMOA-G1-Me.

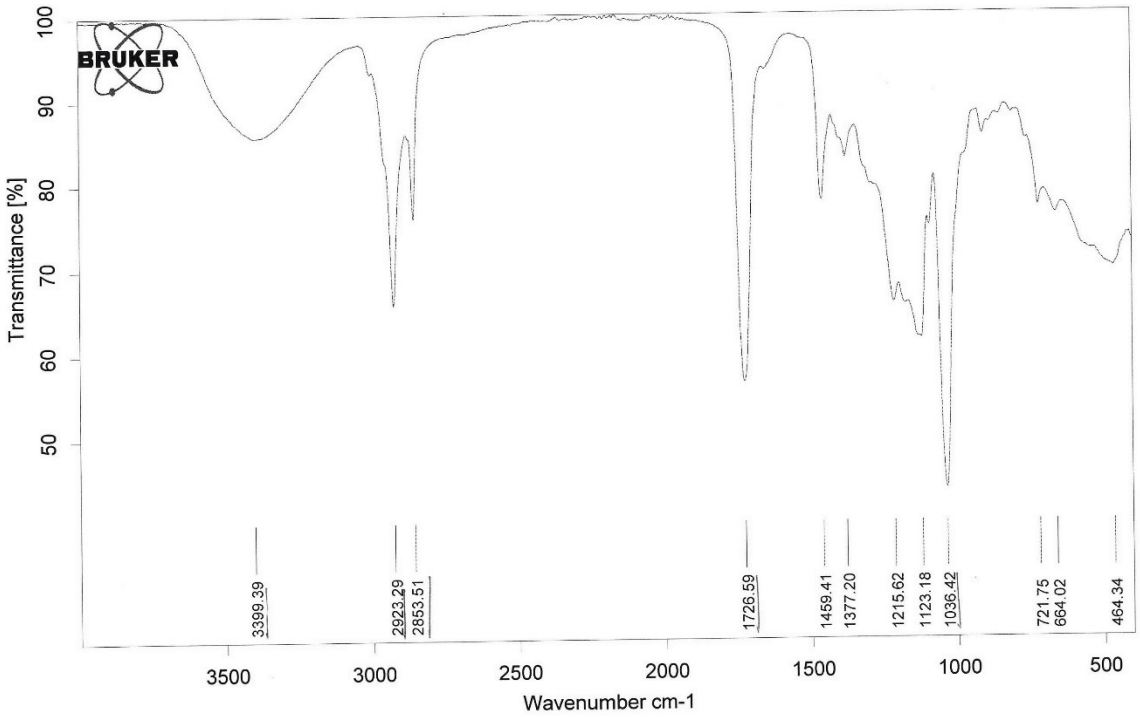


Fig. S41. FT-IR spectra of GMOA-G1-OH.

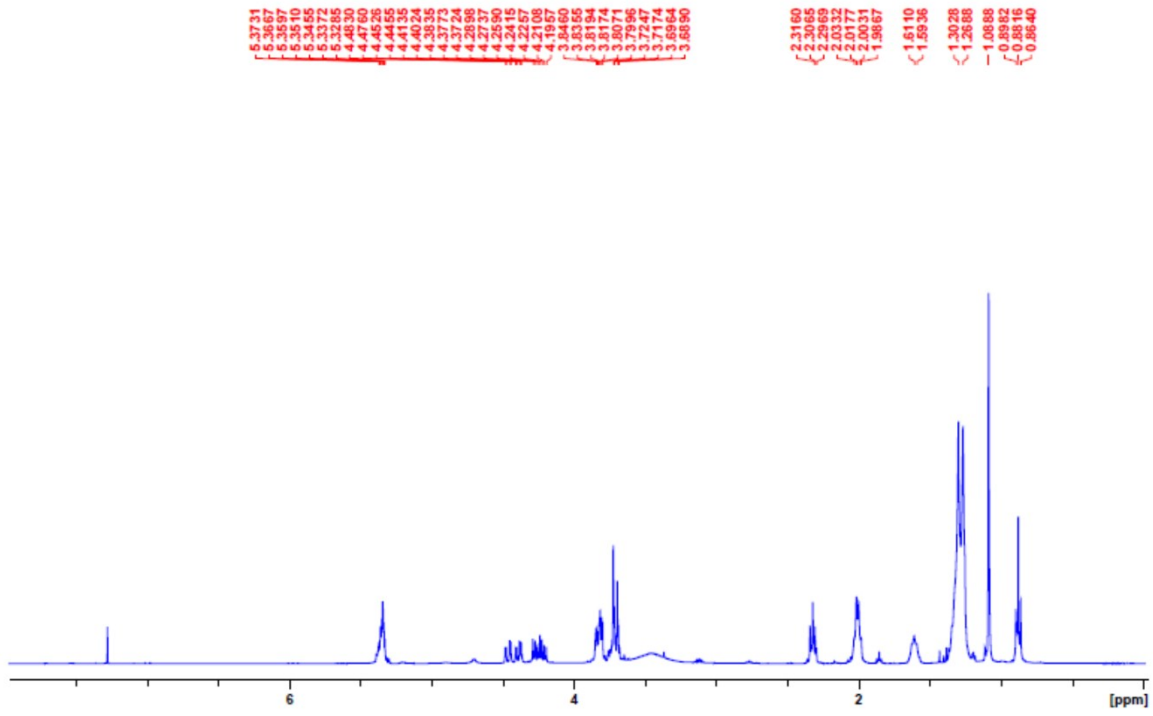


Fig. S42. ^{1}H NMR of GMOA-G1-OH.

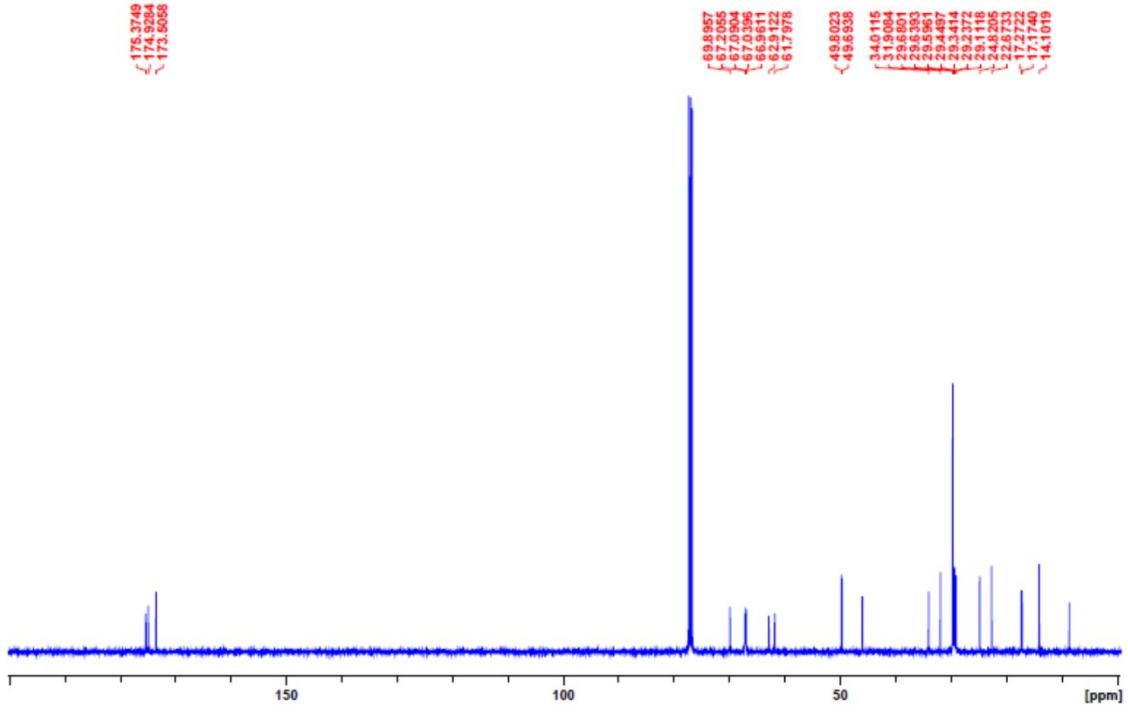
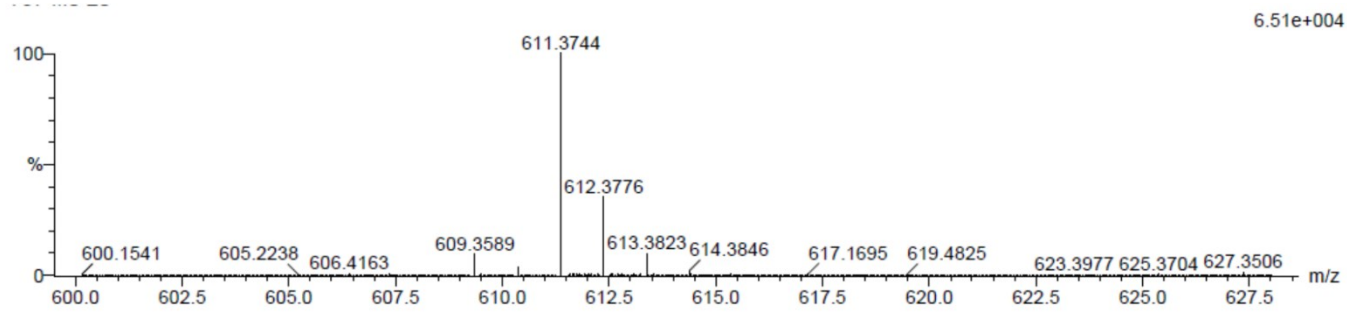


Fig. S43. ^{13}C NMR of GMOA-G1-OH.



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
611.3744	611.3771	-2.7	-4.4	3.5	435.8	0.0	C31 H56 O10 Na

Fig. S44. HRMS of GMOA-G1-OH.

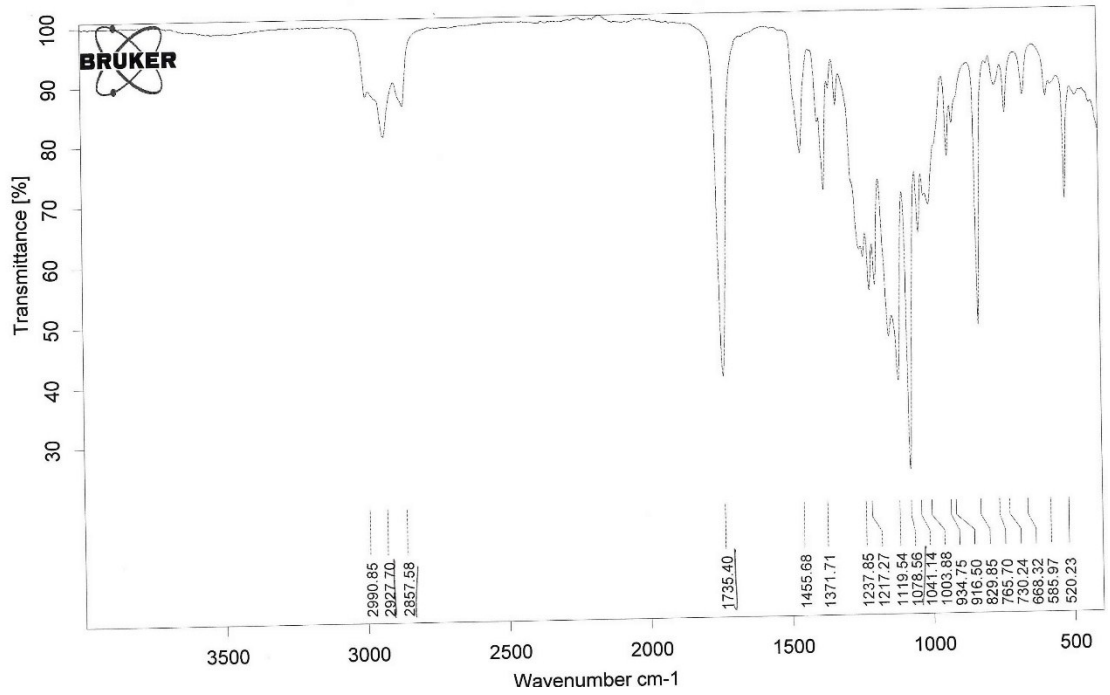


Fig. S45. FT-IR spectra of GMOA-G2-Me.

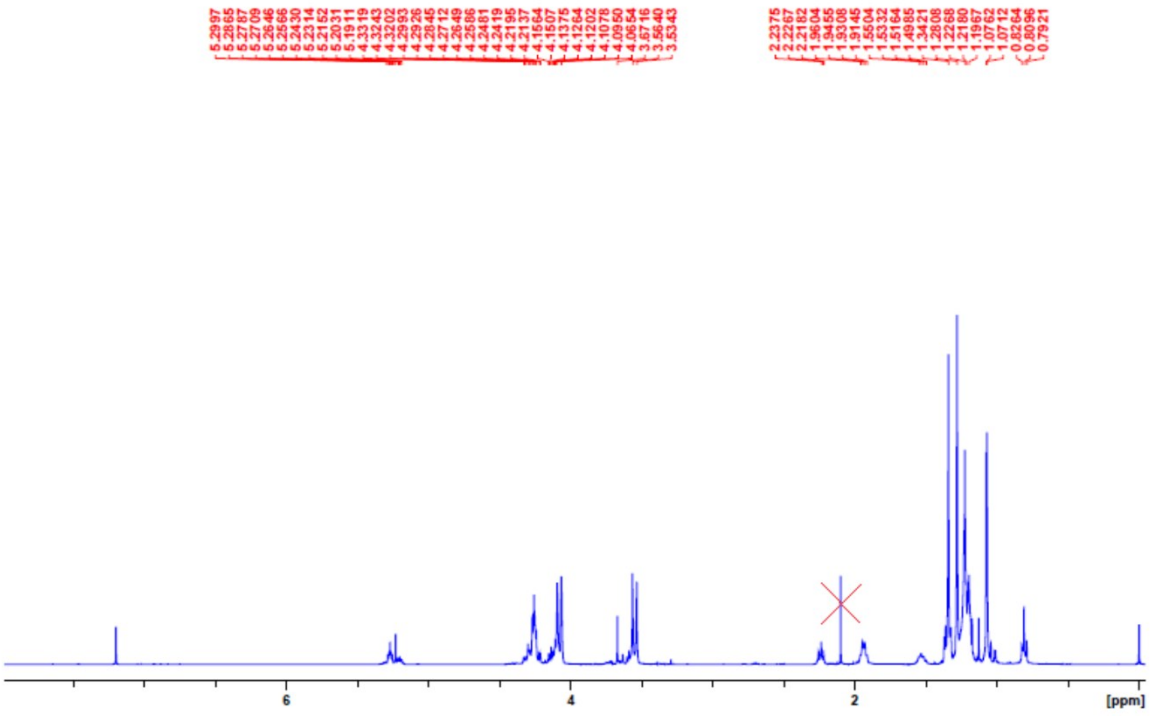


Fig. S46. ^{1}H NMR of GMOA-G2-Me.

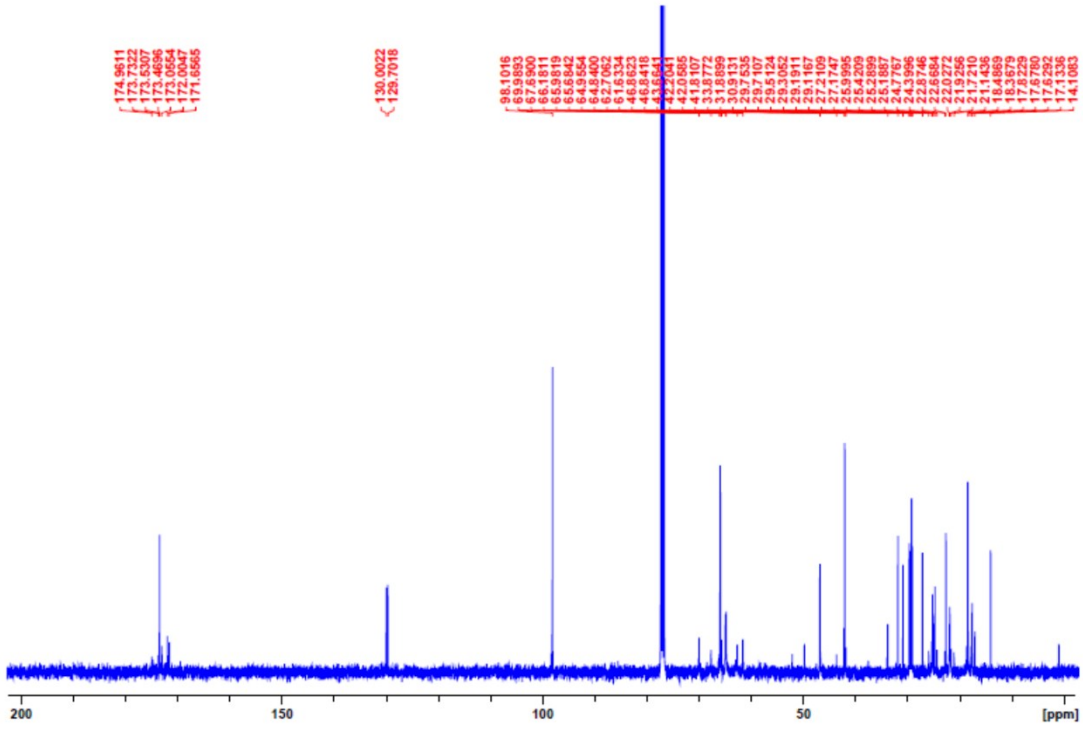


Fig. S47. ^{13}C NMR of GMOA-G2-Me.

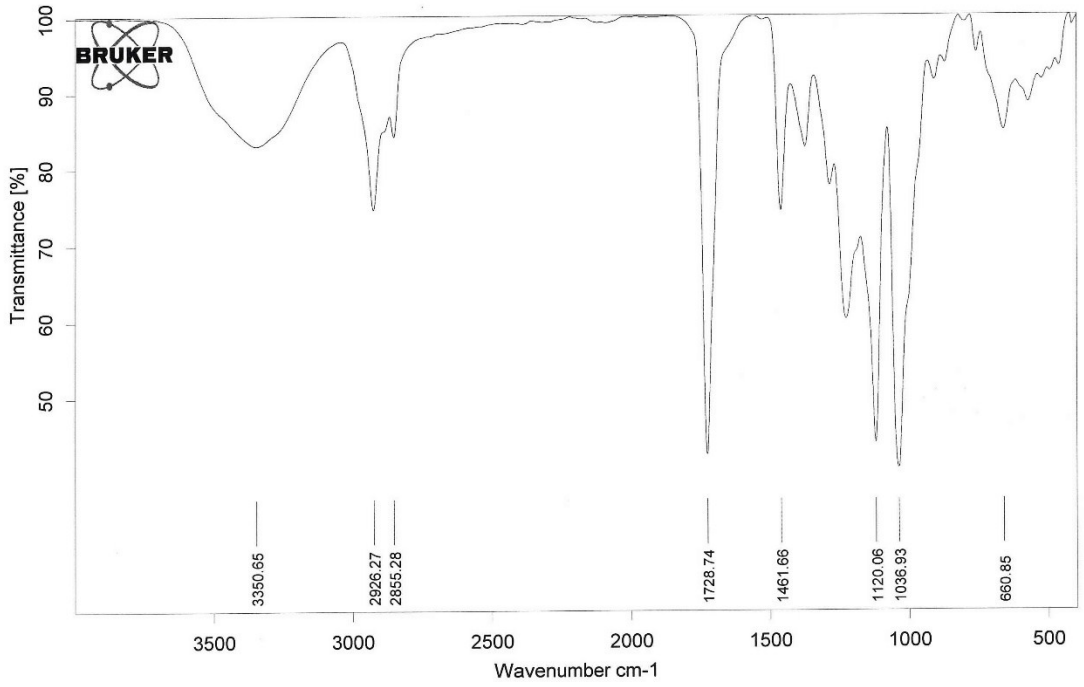


Fig. S48. FT-IR spectra of GMOA-G2-OH.

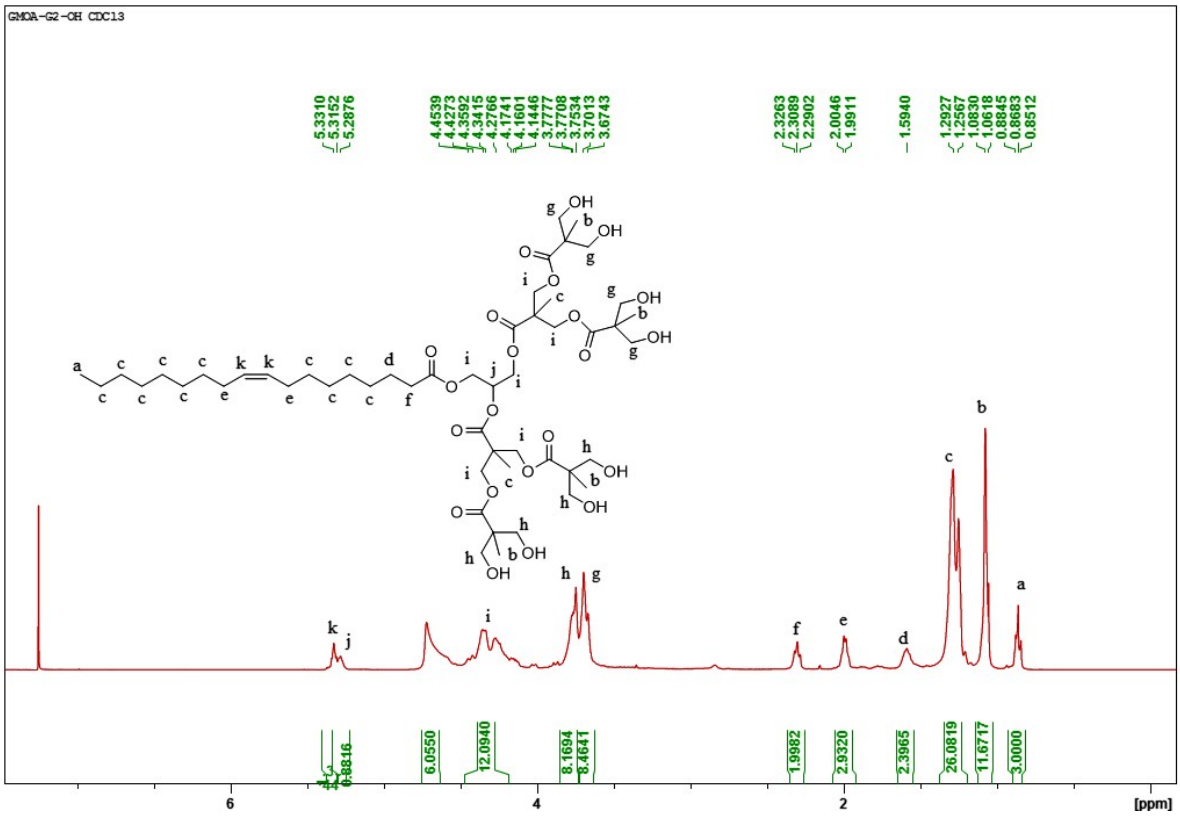


Fig. S49. ^{1}H NMR of GMOA-G2-OH.

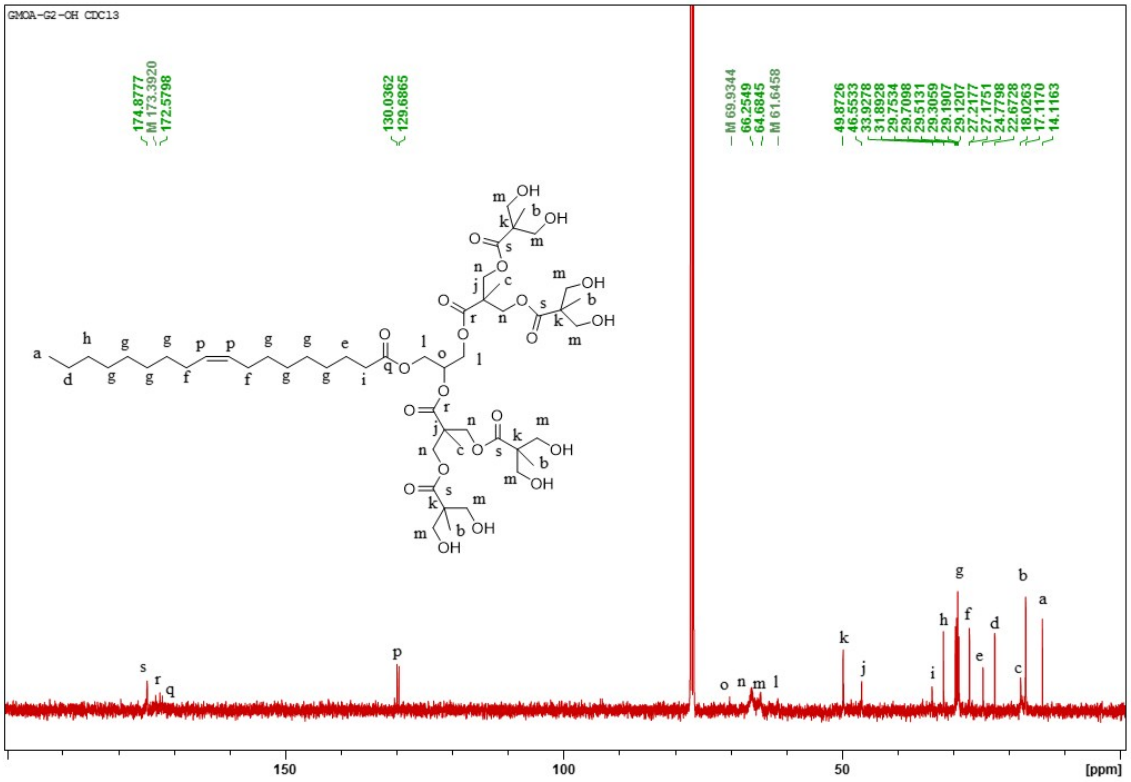
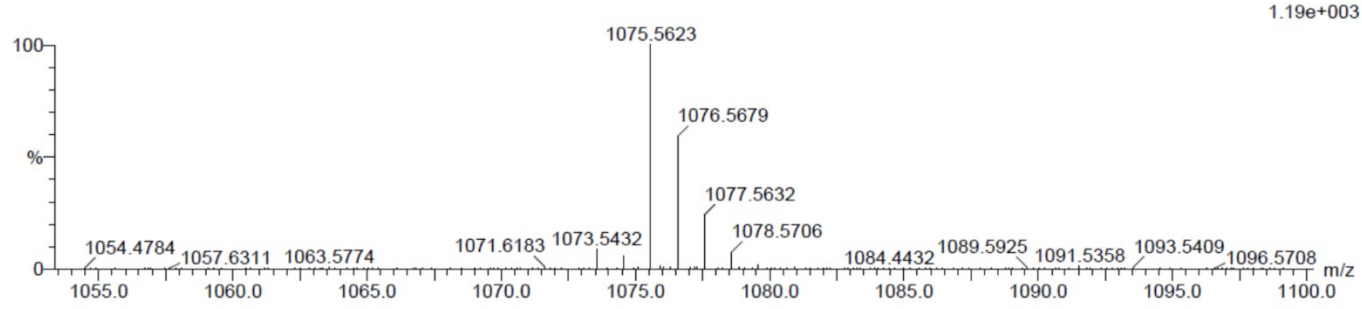


Fig. S50. ^{13}C NMR of GMOA-G2-OH.



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1075.5623	1075.5665	-4.2	-3.9	7.5	39.3	0.0	C51 H88 O22 Na

Fig. S51. HRMS of GMOA-G2-OH.

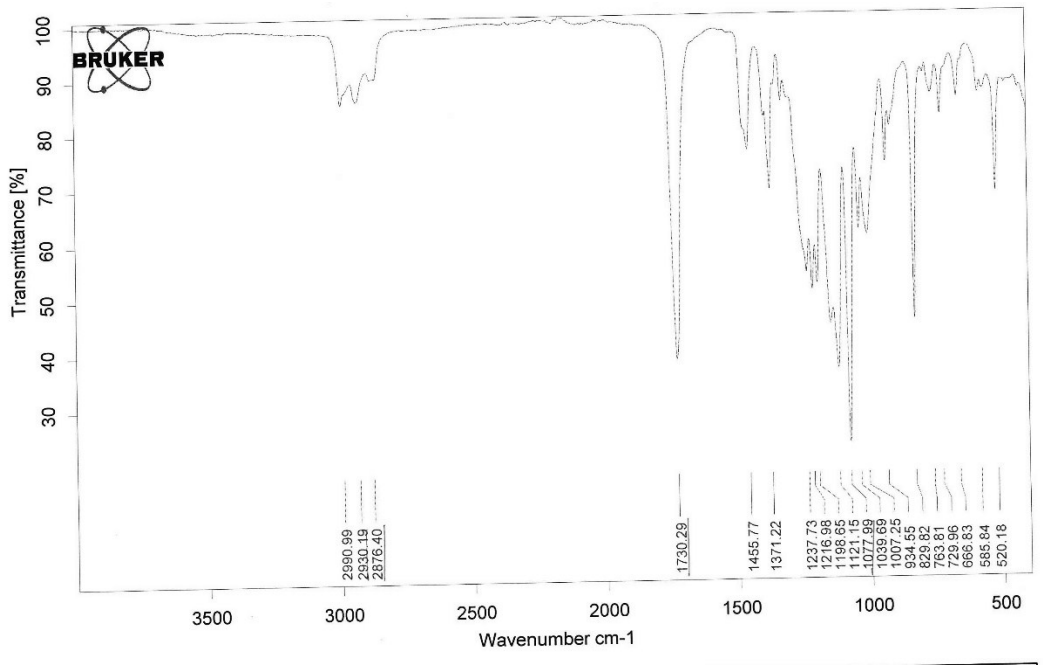


Fig. S52. FT-IR spectra of GMOA-G3-Me.

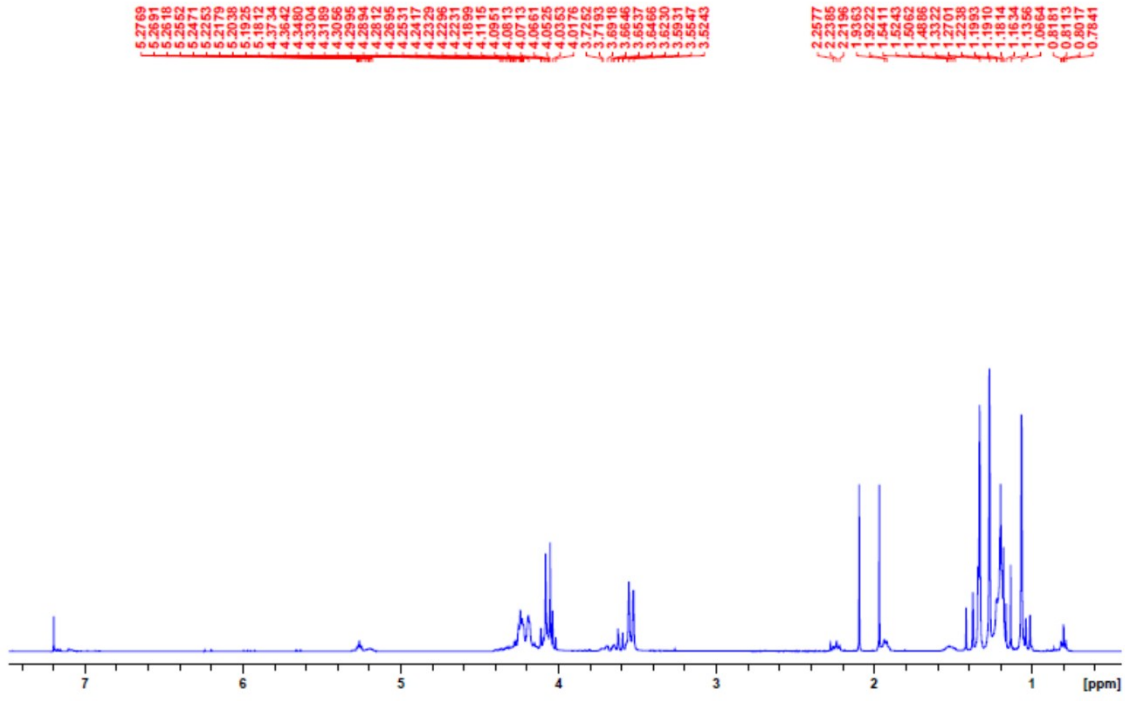


Fig. S53. ^{1}H NMR of GMOA-G3-Me.

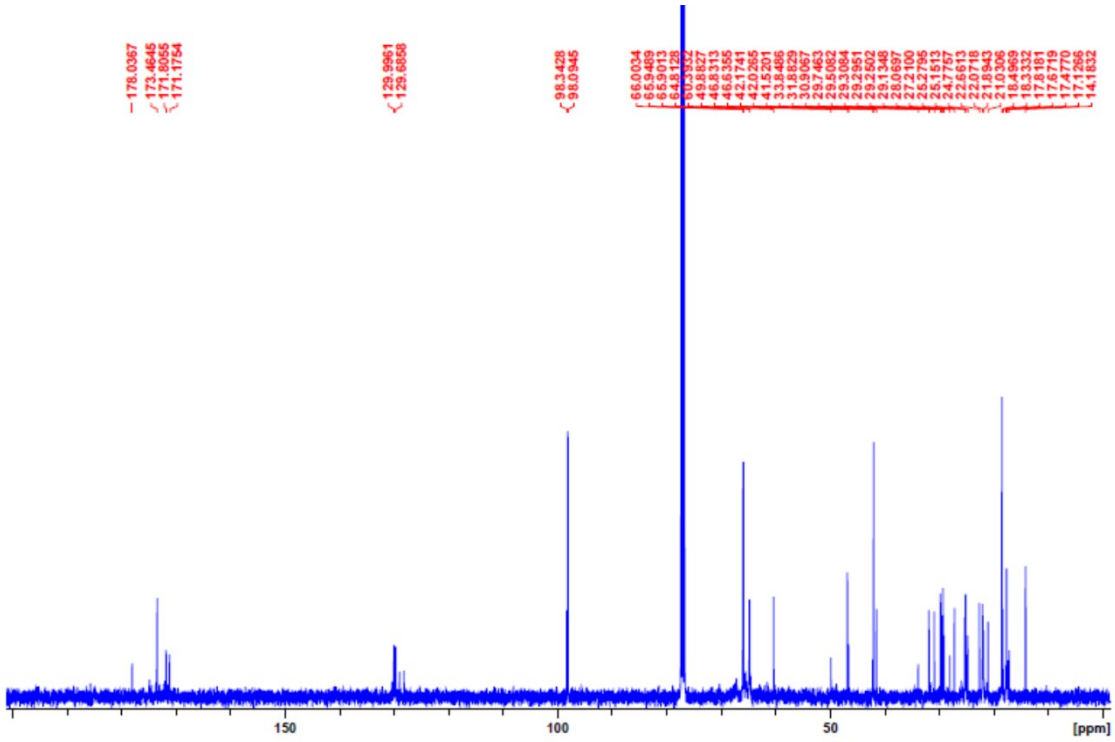
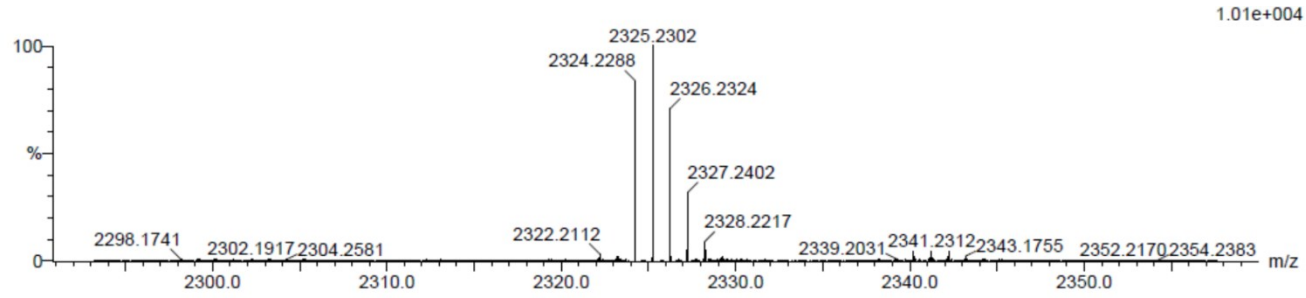


Fig. S54. ^{13}C NMR of GMOA-G3-Me.



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
2324.2288	2324.2320	-3.2	-1.4	22.5	106.2	0.0	C116 H188 O45 Na

Fig. S55. HRMS of GMOA-G3-Me.

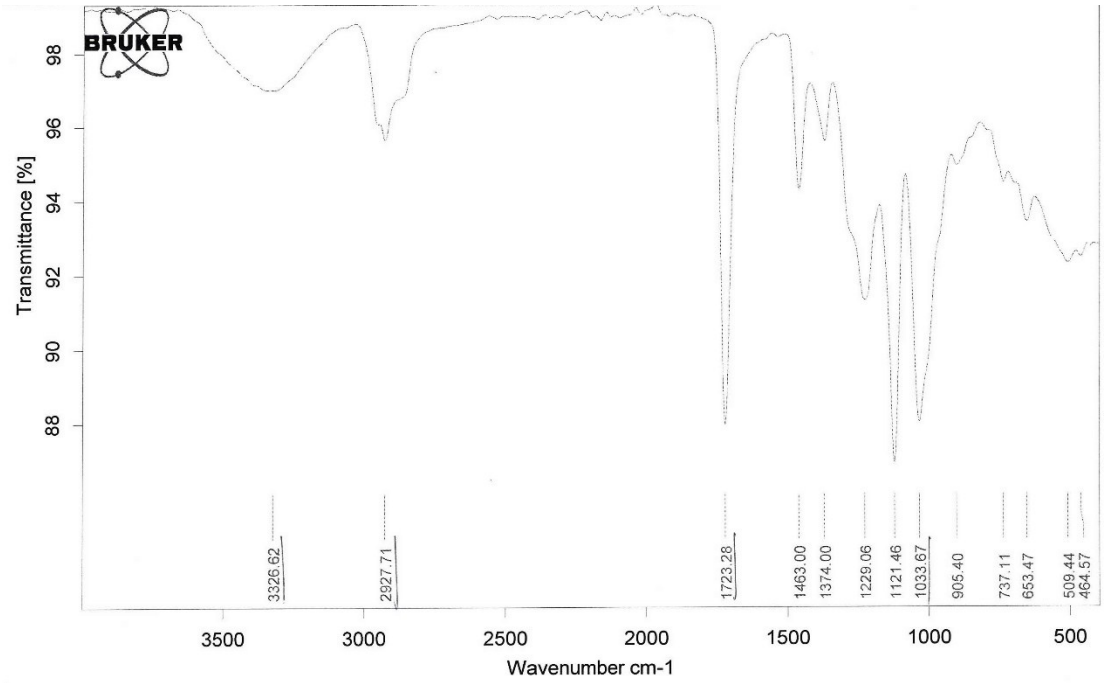


Fig. S56. FT-IR spectra of GMOA-G3-OH.

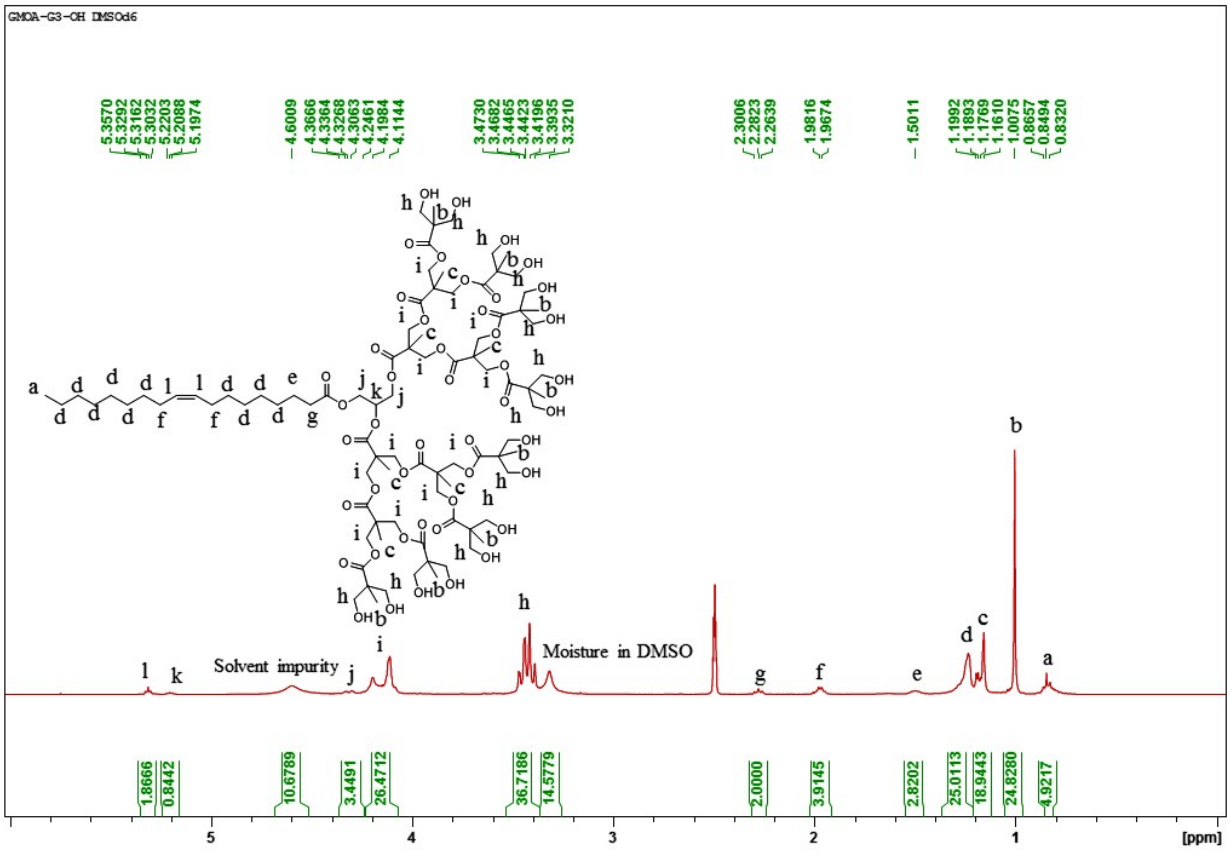


Fig. S57. ^{1}H NMR of GMOA-G3-OH.

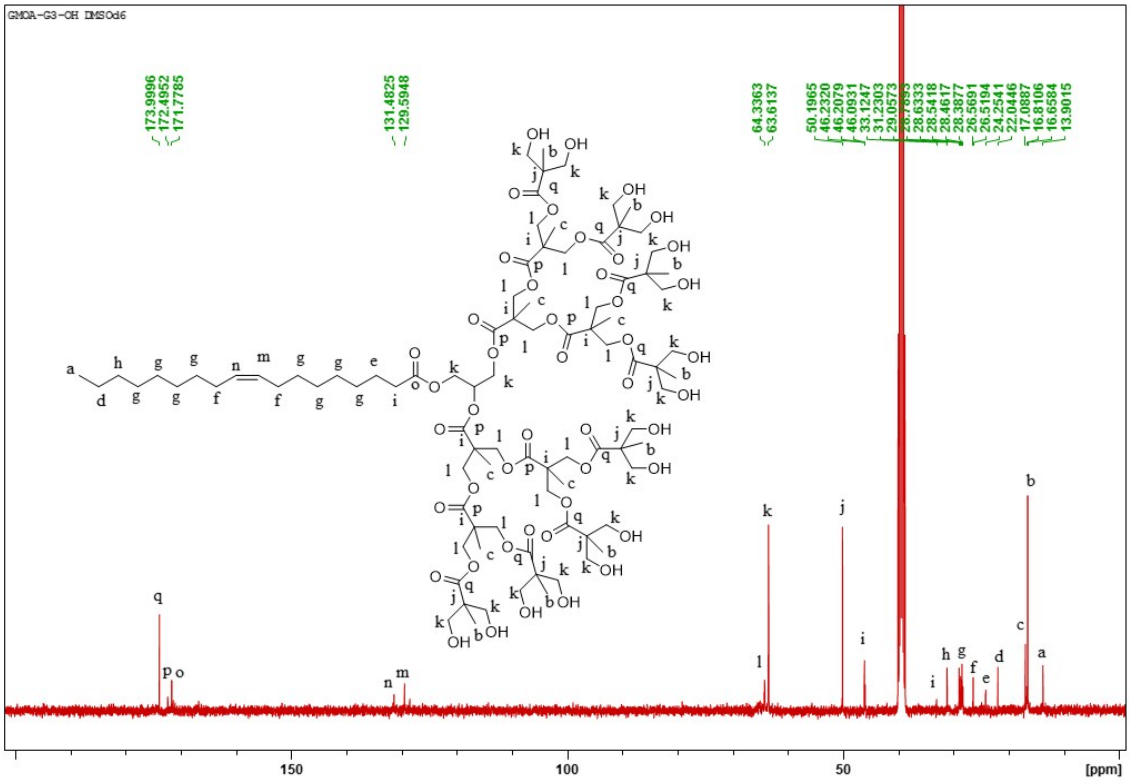
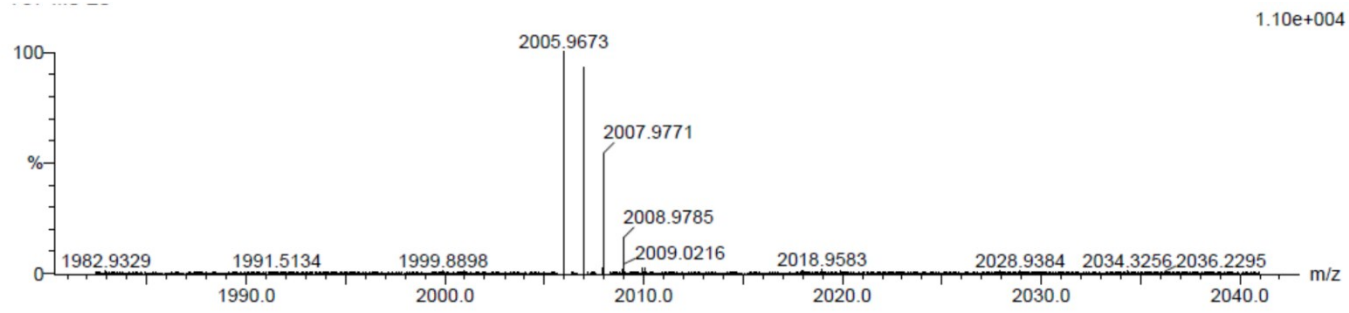


Fig. S58. ^{13}C NMR of GMOA-G3-OH.



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
2005.9673	2005.9609	6.4	3.2	14.5	173.7	0.0	C91 H154 O46 Na

Fig. S59. HRMS of GMOA-G3-OH.

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